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The occurrence of aflatoxin M_1 in industrial and traditional fermented milk: A systematic review study

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Review

Abstract

Aflatoxin M_1 (AFM_1) is a toxic secreted into the milk of animals fed with diets contaminated by aflatoxin B_1, which can cause some adverse health effects in humans. The occurrence of AFM_1 in dairy products varies based on several factors, including the fermentation process. In this article, the published citations from January 2000 to October 2020 regarding the AFM_1 occurrence in industrial and traditional fermented milk were systemically reviewed. According to the findings, a reducing trend in the AFM_1 contamination of fermented milk was observed over the years, mainly in traditional products. Despite this trend, further control measures besides the preventative approaches are needed to deal with the high levels of AFM_1 in fermented milk.

Keywords: AFM_1; yogurt; fermented milk; occurrence; contamination; food safety; traditional dairy products

Introduction

Aflatoxins are toxic, secondary metabolites synthesized by some fungi species in the genus *Aspergillus*, mainly those belonging to the species *A. flavus*, *A. nomius*, and *A. parasiticus* (Ismaiel *et al.*, 2020). Aflatoxins are considered the most important mycotoxins, given their carcinogenic and hepatotoxic effects on animals and humans (Bhat *et al.*, 2010). Among several types of aflatoxins, the most frequent ones found as natural contaminants of foodstuffs are aflatoxins B_1 (AFB_1), B_2 (AFB_2), G_1 (AFG_1), and G_2 (AFG_2) (Nejad *et al.*, 2019). While AFB_1 possesses the highest toxicity, this toxin is also classified as a Group 1 carcinogen by the International Agency for Research on Cancer (2002). In addition, AFM_1 and AFM_2 are produced by hepatic biotransformation of AFB_1 and AFB_2, respectively, and maybe shed through the urine and milk of animals (Campagnollo *et al.*, 2016; Imamura *et al.*, 2015).

Milk and milk products have high nutritional and biological value, contributing to a balanced diet for human beings. Among dairy products, fermented milk is important, as it is consumed by a wide range of people, from infants to elders (Barukcic *et al.*, 2018). Some fermented milk has, in their composition, probiotics that lead to improved digestibility, besides some other health-promoting factors, such as bioactive peptides and bacteriocins (Black, 2011). As AFM_1 is highly stable through pasteurization, ultra-high temperature processing, and

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other processing methods used in dairy production, the toxin may be found not only in processed milk but also in dairy products (Jalili and Scotter, 2015). Yogurt and other fermented milk products are typically manufactured by fermentation of lactic acid in milk, both traditional and industrialized products with different levels of AFM_1, given the range of pH values and fermentation conditions (Govaris *et al.*, 2002). However, studies related to AFM_1 contents in these fermented products are scarce and controversial (Campagnollo *et al.*, 2016; Mahmood Fashandi *et al.*, 2018; Mousavi Khaneghah *et al.*, 2017). Figure 1 presents an overview processing steps of fermented milk and some relevant points regarding the AFM_1 contamination of these products.

When fermented milk is produced using milk contaminated with AFM_1, the mycotoxins are not eliminated at once, as they are resistant to most processing steps (Behfar *et al.*, 2012). Therefore, to safeguard human health, maximum limits of AFM_1 residues recommended in most countries range from 0 to 1.0 μg/L of milk (Iqbal *et al.*, 2015). In the European Union (EU), the tolerable limit for AFM_1 in milk is no more than 0.05 μg/L (European Commission, 2006), while in the United States and Brazil, a maximum level of 0.5 μg/L is accepted (Agência Nacional de vigilância sanitária, 2011; Food and Drug Administration, 2000).

Besides yogurts, other fermented products are also susceptible to AFM_1 contamination, including traditional ones, such as *Lala*, kefir, and Doogh. *Lala* is traditional African fermented milk produced by natural fermentation or mesophilic cultures (Kuboka *et al.*, 2019). Kefir is a dairy product rich in vitamins, essential amino acids, and minerals, made by fermenting the kefir grains (Gamba *et al.*, 2016). Kefir is the most common probiotic product consumed in Europe and is associated with beneficial health effects related to homeostasis balance (Otles and Cagindi, 2003). Doogh is an Iranian fermented product made from yogurt added with potable water, sodium chloride, and probiotic cultures (Kiani *et al.*, 2018). While several studies have been dedicated for evaluating the AFM_1 levels in milk and other dairy products (Fallah, 2010; Kim *et al.*, 2011; Rahmani *et al.*, 2018), no systematical review was conducted to summarize the findings. Therefore, the current investigation was undertaken to systematically review the literature published in the last 20 years regarding the prevalence of AFM_1 in industrial and traditional fermented milk globally.

Literature Search

A systematic literature search was conducted among some international databases such as PubMed, Science Direct, and Google Scholar (as gray literature) using the following key terms: "aflatoxins" OR "aflatoxin M_1" OR "mycotoxins" AND "Occurrence" OR "Contamination" OR "prevalence" OR "incidence" OR "fermented milk" OR "dairy products" OR "cultured dairy" OR "yogurt" OR "Kefir." All relevant articles published from January 2000 to October 2020 that investigated the prevalence of AFM_1 in fermented milk were retrieved and screened for eligibility. In addition, the reference lists of

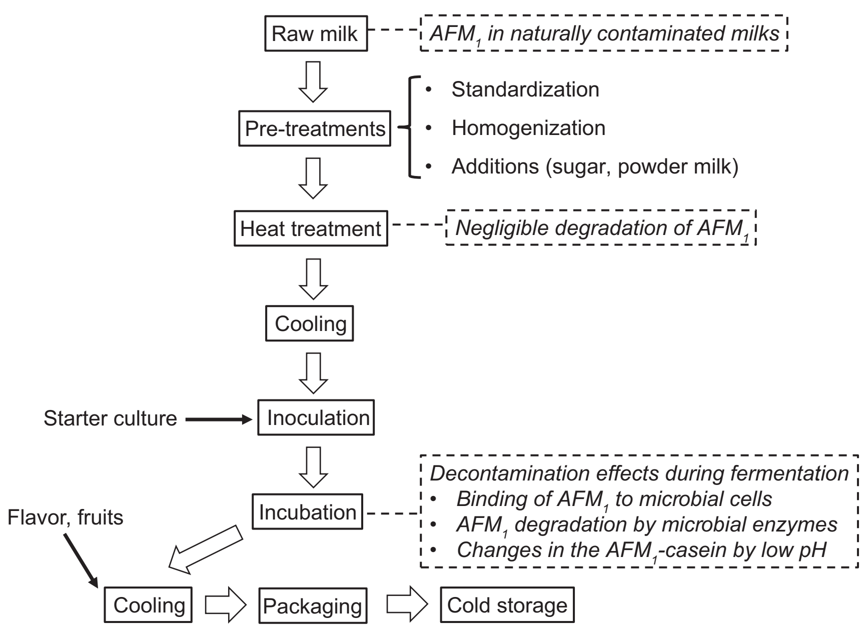

Figure 1. General processing flow chart of fermented milk and relevant steps regarding the aflatoxin M_1 (AFM_1) contamination during manufacture (in italic).

included articles were also manually searched to identify other suitable studies.

After excluding unsuitable articles due to irrelevant content, 150 full texts of potentially eligible articles were downloaded. Then, the downloaded citations were examined for inclusion and criteria of final eligibility. Inclusion criteria were: (1) availability of full-text article, (2) original cross-sectional research studies (not reviews), (3) reporting of AFM_1 prevalence among fermented, milk-based products, (4) indicating an accurate analytical method, and (5) published in the English in order to avoid any mistake during translation from other languages. The citations that did not meet these criteria were excluded. A total of 100 articles were excluded based on the above-mentioned exclusion criteria according to PRISMA, as detailed in Figure 2. Finally, 50 articles that fulfilled the inclusion criteria were included in this review.

The Occurrence of Aflatoxin M_1 in Fermented Milk

Table 1 presents the worldwide prevalence of AFM_1 in yogurt and other fermented milk products during the last 20 years. Several studies reported a high prevalence of AFM_1 in yogurt and other traditional products in African countries. This is consistent with the high prevalence of AFB_1 reported in feedstuff used for dairy cows and AFM_1 in milk in the African continent (Muaz *et al.*, 2021). In addition to the climatic conditions that favor fungal growth in several geographic areas in Africa, the lack of effective regulation of aflatoxins in the food chain and the low public awareness of this risk are among important factors that contribute to high prevalence of aflatoxins in African countries (Wild *et al.*, 2015). In Egypt, 63% of the yogurt samples exceed the EU's AFM_1 levels (Aiad and Aboelmakarem, 2013). The mean prevalence of AFM_1 in Egyptian yogurt samples was higher in the winter than in the summer. Coherently, higher AFM_1 levels in milk samples have also been reported in the winter season in different countries (Bilandzic *et al.*, 2014; De Roma *et al.*, 2017; Fallah, 2010; Ruangwises and Ruangwises, 2010). The reasons for such a higher prevalence of AFM_1 in milk and fermented products during the winter are not well established but may involve higher consumption of AFB_1-contaminated feed by dairy cows during this period, as well as differences in the feed storage and diet composition, and rainfall effects (Fallah, 2010; Hajmohammadi *et al.*, 2020). After incubation of *Lactobacillus acidophilus* and *Bifidobacterium lactis* into the fermented products, a decrease in mycotoxin prevalence was observed at the end of the storage period (Ibrahim *et al.*, 2016). In this regard, the percentages of Egyptian Zabadi yogurt samples exceeding the European limits in 2016 and 2017 were 12.5 and 18.7, respectively. However, these prevalence data were lower in this product than in milk and cheese, mainly in the winter (Ismaiel *et al.*, 2020). In Nigeria, 20

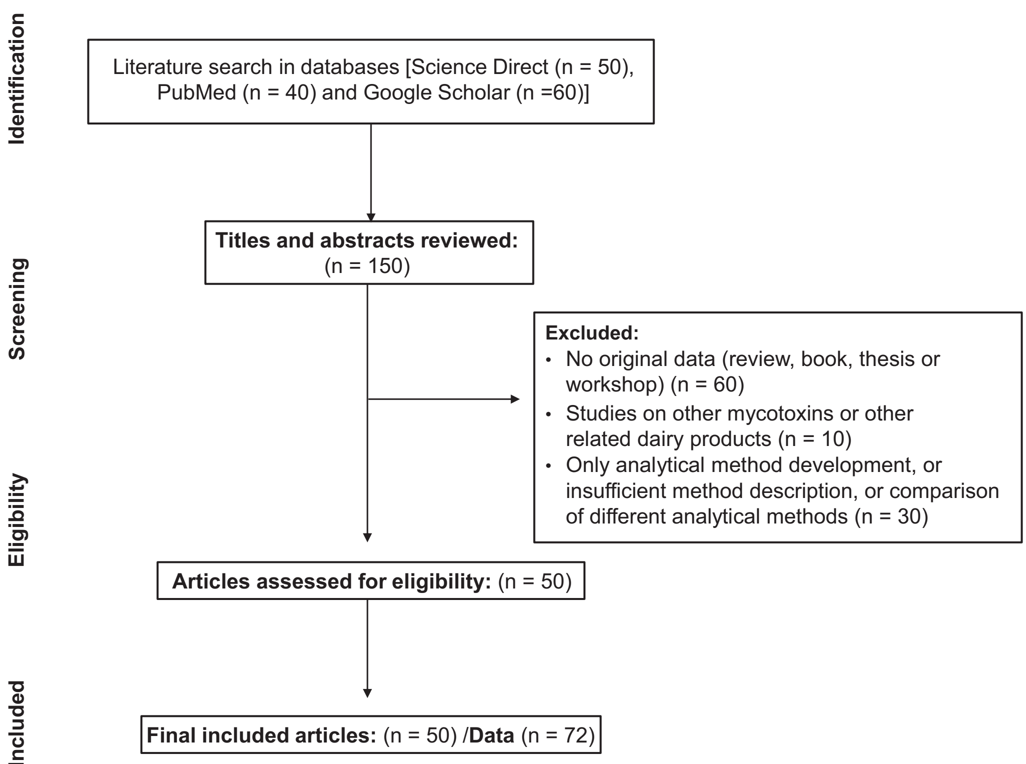

Figure 2. Flow chart describing the search and selection of articles evaluated in the study.

Table 1. **Occurrence of aflatoxin M_1 in yogurt and other fermented milks reported in the last 20 years.**

Country	Type of product	Samples analyzed (n)	Positive samples		LOD (ng/kg or L)	Concentration (ng/kg or L)		Analytical method	Reference
			n	%		Range	Mean		
Africa:									
Egypt	Yogurt[a]	30	8	26	5	11.40–98.80	28.41	ELISA	Aiad and Aboelmakarem (2013)
Egypt (Winter)	Yogurt	24	12	50	NR	56.60–84.14	64.68	HPLC	Ibrahim *et al.* (2016)
Egypt (Summer)	Yogurt	24	12	50	NR	31.46–66.05	39.13	HPLC	Ibrahim *et al.* (2016)
Egypt (2016 production)	Yogurt *Zabady*	32	4	12	50	130–240	185	HPLC	Ismaiel *et al.* (2020)
Egypt (2017 production)	Yogurt *Zabady*	32	6	19	50	100–170	130	HPLC	Ismaiel *et al.* (2020)
Nigeria	Yogurt	2	10	10	1	583.5–647.0	0.62 µg/L	HPLC	Anthony *et al.* (2016)
Burundi	Yogurt	6	6	100	NR	8,200–63,200	33,500	ELISA	Udomkun *et al.* (2018)
Kenya	Yogurt	8	3	37	NR	< LOD—690	NR	ELISA	Langat *et al.* (2016)
Kenya	Yogurt	21	12	57	2	17–1,100	134	ELISA	Lindahl *et al.* (2018)
Kenya	*Lala*	27	8	30	2	12–160	48	ELISA	Lindahl *et al.* (2018)
Kenya	Yogurt	17	13	77	2	26–270	96	ELISA	Lindahl *et al.* (2018)
Kenya	*Lala*	8	5	63	2	10–340	111	ELISA	Lindahl *et al.* (2018)
Kenya	Yogurt	NR	NR	NR	5	NR	379.3	ELISA	Kuboka *et al.* (2019)
Kenya	*Lala*	NR	NR	NR	5	NR	379.3	ELISA	Kuboka *et al.* (2019)
Congo Republic	Yogurt	2	3	67	NR	4,800–26,000	16,100	ELISA	Udomkun *et al.* (2018)
Americas:									
Brazil	Yogurt	53	47	72	3	10–529	NR	HPLC	Iha *et al.* (2011)
Brasil	Yogurt	3	3	100	3	75.1–112.9	94	HPLC	Iha *et al.* (2013)
Asia:									
Qatar	Yogurt	21	16	76	NR	4.16–38.21	31.32	ELISA	Hassan *et al.* (2018)
China	Yogurt	27	15	55	5	4.0–47.0	17.2	HPLC	Guo *et al.* (2019)
China	Yogurt	NR	NR	NR	0.6	NR	NR	ELISA	Zhou *et al.* (2019)
South Korea	Yogurt	55	15	27	20	20–150	51	HPLC	Yoon *et al.* (2016)
South Korea	Yogurt	60	50	83	2	3–172	29	ELISA	Kim *et al.* (2000)

(continues)

Table 1. Continued

Country	Type of product	Samples analyzed (n)	Positive samples		LOD (ng/kg or L)	Concentration (ng/kg or L)		Analytical method	Reference
			n	%		Range	Mean		
Iran	Pasteurized yogurt	40	40	100	NR	2.1–61.7	15.1	ELISA	Barjesteh *et al.* (2010)
Iran	Yogurt	10	10	100	NR	7–53	25	ELISA	Barjesteh *et al.* (2010)
Iran	Yogurt	68	45	66	12	15–119	32	HPLC	Fallah (2010)
Iran	Traditional yogurt	60	14	23	12.5	15–36	17	HPLC	Fallah *et al.* (2011)
Iran	Industrial yogurt	61	30	49	12.5	15–102	26	HPLC	Fallah *et al.* (2011)
Iran	Traditional Doogh	65	9	14	12.5	13–29	NR	HPLC	Fallah *et al.* (2011)
Iran	Industrial Doogh	71	16	22.5	12.5	13–53	NR	HPLC	Fallah *et al.* (2011)
Iran	Yogurt	60	48	80	10	19.7–319.4	130.5	ELISA	Rahimi (2012)
Iran	Yogurt	60	59	98	NR	6.2–87	51.7	ELISA	Issazadeh *et al.* (2012)
Iran	Yogurt	13	13	100	NR	5–36	13.5	ELISA	Arast *et al.* (2012)
Iran	Yogurt	40	14	35	NR	11.4–115.8	130.5	ELISA	Nilchian and Rahimi (2012)
Iran	Yogurt	80	77	96	5	< LOD—100	29.1	ELISA	Mason *et al.* (2015)
Iran	Yogurt	42	10	24	1	6.3–21.3	15.1	ELISA	Bahrami *et al.* (2015)
Iran	Doogh	44	6	14	NR	7.0–12.1	9.0	ELISA	Bahrami *et al.* (2015)
Iran	Yogurt	90	90	100	NR	5.0–83.0	32.1	ELISA	Nikbakht *et al.* (2016)
Iran	Yogurt	18	15	83	NR	7.8–12.1	10.3	ELISA	Sohrabi and Gharahkoli (2016)
Iraq	Yogurt	32	32	100	NR	0.16–42.74	16.92	ELISA	Najim and Jasim (2014)
Iraq	Traditional yogurt	20	15	75	NR	22.2–172.9	103.9	HPLC	Mossawei *et al.* (2016)
Iraq	Yogurt	20	10	50	NR	30.5-107.4	58.37	HPLC	Mossawei *et al.* (2016)
Kuwait	Yogurt	2	1	50	10	NR	NR	HPLC	Ivastava *et al.* (2001)
Lebanon	Yogurt	64	21	33	5	5–50	NR	ELISA	El Khoury *et al.* (2010)
Lebanon	Yogurt	NR	NR	72	NR	NR	24.55	ELISA	Hassan and Kassaify (2014)
Lebanon	Yogurt	28	18	64	3.2	15–545	91	HPLC	Daou *et al.* (2020)
Lebanon	Strained yogurt	27	24	89	3.2	37–1,843	201	HPLC	Daou *et al.* (2020)
Lebanon	Yogurt *Ayran*	9	8	89	3.2	20–315	242	HPLC	Daou *et al.* (2020)
Malaysia	Yogurt	5	2	40	2	7.5–31	25.4	ELISA	Nadira *et al.* (2016)

Pakistan	Yogurt	96	59	61	4	4.0–615.8	90.4	HPLC	Iqbal and Asi (2013)
Pakistan (Winter)	Yogurt	51	13	25	4	NR	53	HPLC	Iqbal *et al.* (2013)
Pakistan (Summer)	Yogurt	45	8	18	4	NR	19	HPLC	Iqbal *et al.* (2013)
Pakistan (Winter)	Plain yogurt	36	15	42	0.4	NR	63.6	HPLC	Iqbal *et al.* (2017)
Pakistan (Winter)	Flavored yogurt	30	17	57	0.4	NR	50.5	HPLC	Iqbal *et al.* (2017)
Paquistão (Summer)	Plain yogurt	30	11	37	0.4	NR	59.6	HPLC	Iqbal *et al.* (2017)
Pakistan (Summer)	Flavored yogurt	25	10	40	0.4	NR	45.3	HPLC	Iqbal *et al.* (2017)
Taiwan	Yogurt	24	3	12	5	7–44	NR	HPLC	Lin *et al.* (2004)
Turkey	Yogurt	40	32	80	50	61.61–365.64	NR	ELISA	Gurbay *et al.* (2006)
Turkey	Plain yogurt	104	68	65	NR	1–100	NR	ELISA	Akkaya *et al.* (2006)
Turkey	Yogurt with fruits	21	7	33	NR	1–100	NR	ELISA	Akkaya *et al.* (2006)
Turkey	Yogurt	52	29	56	NR	1–150	NR	ELISA	Akkaya *et al.* (2006)
Turkey	Yogurt	80	70	87	5	10–475	66.1	ELISA	Atasever *et al.* (2011)
Turkey	Yogurt *Ayran*	80	72	90	5	6–264	36.5	ELISA	Atasever *et al.* (2011)
Turkey	Yogurt	50	10	20	2	40.62–72.04	55.28	ELISA	Temamogullari and Kanici (2014)
Turkey	Yogurt	19	17	89	100	16–107.2	47.92	HPLC	Sarica *et al.* (2014)
Turkey	Yogurt	60	2	3	5	24–28	NR	HPLC	Sahin *et al.* (2016)
Turkey	Yogurt *Ayran*	60	1	2	5	NR	5	HPLC	Sahin *et al.* (2016)
Europe:									
Italy	Yogurt	120	73	61	1	1.0–32.2	9.1	HPLC	Galvano *et al.* (2001)
Portugal	Plain yogurt	48	2	4	10	43.0–45.0	44.0	HPLC	Martins and Martins (2004)
Portugal	Yogurt with fruits	48	16	33	10	19.0–98.0	51.12	HPLC	Martins and Martins (2004)
Serbia	Fermented milks	302	NR	NR	6	25–500	190	ELISA	Keskic *et al.* (2016)
Spain	Yogurt	72	2	3	25	NR	38.34	ELISA	Cano-Sancho *et al.* (2010)
Spain	Yogurt	6	2	33	25	NR	21.6	ELISA	Cano-Sancho *et al.* (2015)

[a]Without any further designation, the term “yogurt” applies to industrial products.
ELISA: enzyme-linked immunosorbent assay; HPLC: high-performance liquid chromatography; LC-MS/MS: liquid chromatography coupled to tandem mass spectrometry. NR: not reported.

samples of yogurt were analyzed, and 10% were contaminated with AFM_1 (Anthony *et al.*, 2016). In a study carried out in Nairobi, the capital of Kenya, AFM_1 was analyzed in samples of fermented milk and yogurt, and contamination was observed in levels above 0.05 μg/L (Langat *et al.*, 2016). In Dagoretti and the Westland area belonging to Nairobi, 77 and 57% of *Lala* and yogurt, respectively, contained detectable levels of AFM_1 (Lindahl *et al.*, 2018). In Nairobi, a study with pasteurized yogurt and *Lala* revealed that all samples had AFM_1 above the detection limit (5ng/kg). After undergoing an additional experimental fermentation, both products showed a significant reduction in AFM_1 prevalence (Kuboka *et al.*, 2019). The prevalence of AFM_1 in yogurt and milk samples was evaluated in Burundi in the Republic of the Congo, and 29% of them showed levels much higher than the limits recommended by the EU (Udomkun *et al.*, 2018).

Few studies considering the prevalence of AFM_1 in fermented milk produced in the Americas and European countries were conducted (Table 1). In Brazil, 95% of the samples of yogurt or dairy-based drinks from the Ribeirão Preto region were contaminated with AFM_1 (Iha *et al.*, 2011). Interestingly, while the naturally contaminated yogurts from were incubated for 12 h, there was a reduction about 6% in the toxin levels (Iha *et al.*, 2013). The fermentation process in yogurts contributes to reducing the concentration of AFM_1 due to factors such as low pH, production of organic acids, and the presence of bacteria that synthesize lactic acid and other byproducts of fermentation (Govaris *et al.*, 2002).

As for the European countries, AFM_1 was detected in the Cataluña region of Spain among 2.8% of the samples analyzed, the only one region that showed contamination above that determined by the EU (Cano-Sancho *et al.*, 2010). In another study, however, 33% of yogurt samples from the same Spanish region were contaminated with AFM_1, with none of them exceeding the European limits (Cano-Sancho *et al.*, 2015). In samples of yogurt from Italian supermarkets analyzed in 1996, 61% showed levels of AFM_1, but similar to Spain, none of them exceeded the limits determined by the EU (Galvano *et al.*, 2001). In a study carried out in Portugal, 4.2% of the samples of plain yogurt and 33.3% of the samples of strawberry yogurt were contaminated with this toxin (Martins and Martins, 2004). In Serbia, the mean concentrations of AFM_1 in dairy products and fermented dairy drinks in 2015 were 0.018 and 0.019 μg/kg, respectively, with 5.86 and 2.64% of the samples exceeding the limits determined by the EU. It was also observed that the toxin levels were more significant in the winter and autumn in both products (Keskic *et al.*, 2016).

The majority of data describing the prevalence of AFM_1 in fermented milk were provided by studies in Asian countries (Table 1). In Qatar, the incidence of AFM_1 in yogurts was analyzed using an immunoenzymatic assay (ELISA); 76.1% of the samples were positive. However, none of them showed contamination levels above the EU maximum limits, posing no public health threats in this country (Hassan *et al.*, 2018). AFM_1 prevalence in yogurts produced with buffalo milk in different dairy factories in Southern China were evaluated, and none of the samples had levels greater than the limit of 500 ng/kg determined in the country(Guo *et al.*, 2019). Another study carried out with cow milk showed AFM_1 levels inside the limit determined by this country and the EU (Zhou *et al.*, 2019). In South Korea, 27.27% of the yogurt samples showed AFM_1, but none of them was above the limit determined by the Korean Ministry of Food and Drug Safety (0.5 μg/kg) (Kim-Soo *et al.*, 2016). However, in a previous study, 83% of yogurt samples were contaminated by this toxin (Kim *et al.*, 2000).

In the Mazandaran province of Iran, 100% of the pasteurized yogurt and local yogurt samples were contaminated with AFM_1 (Barjesteh *et al.*, 2010). However, in another study in Iran, 20.6% of yogurt samples were contaminated with levels above the limits determined by the local regulations (0.05 μg/L) and were greater in the winter than the summer (Fallah, 2010). Moreover, samples of traditional and industrial yogurt and Doogh were evaluated, and the AFM_1 incidences in both these industrial products were greater in the autumn and winter than in traditional ones. As for Doogh samples, the contamination levels were low, and no significant seasonal effect was observed (Fallah *et al.*, 2011). Seasonal factors may influence the presence of the toxin in these products, as some studies observed higher levels of contamination in milk samples in the autumn and winter compared with summer and spring (Kamkar, 2005). These variations may be related to the procedures during processing, degree of milk contamination, type of yogurt, fermentation conditions, geographic regions, season, country, and analytical methods used to detect these toxins (Di Guan *et al.*, 2011). In general, yogurts have shown lower contamination levels with AFM_1 than cheese (Rabie *et al.*, 2019), as the fermentation process contributes to reducing the concentration of AFM_1 because of low pH and the production of fermentation-related byproducts such as organic acids, including lactic acid, among other factors (Campagnollo *et al.*, 2016). In milk, AFM_1 binds to casein, and the modifications on its structure caused by pH reduction during fermentation may lead to changes in this bound (Govaris *et al.*, 2002). However, the exact mechanisms involved in the mycotoxin decontamination during the fermentation process are not entirely understood. Several experimental data indicate that aflatoxin reduction in fermented products occurs through its binding to the cell wall components of starter cultures, as reviewed by Muaz *et al.* (2021), or through degradation of the toxins by microbial enzymes into less toxic substances (Guo *et al.*, 2020). The most studied bacteria with practical AFM_1-binding abilities are lactic acid bacteria belonging to the genus *Levilactobacillus* spp. (former *Lactobacillus* sp.) such as *L. rhamnosus* and *L. plantarum* (Sadiq *et al.*, 2019). Regarding bio-detoxification, several species in the genera *Pseudomonas*, *Rhodococcus*, *Streptomyces*, *Bacillus*, and *Pleurotus* have been reported to be capable of degrading aflatoxins (Guo *et al.*, 2020). However, these bacterial species are not allowed to be used as starter cultures in fermented foods. The combination of fermentation with some emerging technologies, such as ultrasound, ohmic heating, and cold plasma, has been proposed, aiming at improving aflatoxin's detoxification (Gavahian *et al.*, 2021).

In Shahr-e Kord, Iran, AFM_1 was detected in 35% of the yogurt samples, but not above the EU's acceptable limit (Nilchian and Rahimi, 2012). In Gilan, another province of Iran, 63.33% of the yogurt samples were above the EU limits (Issazadeh *et al.*, 2012). In central Iran, yogurt samples showed mean AFM_1 contamination levels of 13.55 ng/kg (Arast *et al.*, 2012). In Isfahan, 80% of the yogurt samples were contaminated with this toxin, and 5% of them were above the limit determined by the EU (Rahimi, 2014). In traditional Iranian yogurts, these toxin levels were more significant than in industrialized products (Mason *et al.*, 2015). Still, in Iran, aflatoxins levels were evaluated in yogurt and Doogh samples, with 23.8 and 13.6%, respectively, yielding positive results (Bahrami *et al.*, 2016). However, in Iran, 100% of the yogurt samples collected in 2014 were contaminated, with 22.22% above the AFM_1 limits determined by the EU (Nikbakht *et al.*, 2016). On the other hand, 83.3% of the yogurt samples were positive for AFM_1 in another study, although none of them was above the limits determined by the Institute of Standards and Industrial Research of Iran (50 ng/L) (Sohrabi and Gharahkoli, 2016).

In Bagdad, the capital of Iraq, 100% of the yogurt samples from supermarkets were contaminated with AFM_1 (Jasim and Najim, 2014). A study carried out with local and imported yogurts in Iraq found that 75 and 50%, respectively, of the samples contained AFM_1 (Al-Mossawei *et al.*, 2016). In Kuwait, one sample out of two yogurt samples produced in a local farm was contaminated with AFM_1 (Ivastava *et al.*, 2001). In Lebanon, 32.81% of the samples analyzed showed the presence of AFM_1, with 6.25% of them exceeding the limits determined by the EU (El Khoury *et al.*, 2011). Still, in Lebanon, 72% of the yogurt samples analyzed showed AFM_1, with 13% above the recommended limits (Hassan and Kassaify, 2014). In another study carried out in Lebanon with different yogurt types, it was observed that 64.3% of the samples were positive for AFM_1, and 35.7% were above the limits recommended by the EU. Strained yogurt, popularly consumed by the Lebanese population, showed 88.9% contaminated samples, with 81.5% above the EU acceptable limits. The authors suggested that these findings may be due to low-quality powdered milk in the production, leading to high levels of contamination in the final product. As for the yogurt drink Ayran, 88.9% of the samples were positive, with 44.4% above the EU recommended limits (Daou *et al.*, 2020).

In Malaysia, 40% of the yogurt samples collected in January 2014 were contaminated with AFM_1, although none of them was above the limits determined by the EU (Nadira *et al.*, 2017). A study carried out in the winter and summer in Pakistan showed that 37 and 29% of the samples of yogurt, respectively, were contaminated with this toxin, and were above the country limits (0.05 μg/L) (Iqbal *et al.*, 2013). In Punjab, a province of Pakistan, 47% of the yogurt samples were above the legal limits (Iqbal and Asi, 2013). Corroborating these findings, another study carried out in the winter and summer showed that plain yogurt and flavored yogurt samples were contaminated with AFM_1 by 20 and 16%, respectively, and were above the levels determined by the EU during the summer. In the winter, 27.7 and 40%, respectively, were above the EU limits, posing a considerable threat to the population's health (Iqbal *et al.*, 2017). In Taiwan, 12.5% of the samples of yogurt beverages were contaminated with AFM1 but at low levels (Lin *et al.*, 2004).

On the other hand, in Ankara, Turkey's capital, 32% of the yogurt samples showed AFM_1 levels above the country's limit (Gurbay *et al.*, 2006). Also, in Turkey, 11.53% of the yogurt samples, 9.52% of fruit yogurt samples, and 21.15% of strained yogurt samples showed AFM_1 levels greater than those allowed by the existing regulations in the country (50 ng/kg) (Akkaya *et al.*, 2006). Corroborating this finding, 20% of the yogurt samples evaluated in other studies showed contamination levels above the acceptable limits by Turkish Food Codex (2008) (50 ng/kg) (Atasever *et al.*, 2011; Temamogullari and Kanici, 2014). Another study in Ankara showed that 89.5% of the yogurt samples were contaminated with AFM_1. Only 5 were above the limit determined by the local regulations (Sarica *et al.*, 2015). On the other hand, in Turkey, only two yogurt samples and one sample of Ayran showed AFM_1, but the levels were below the EU limits (Sahin *et al.*, 2016).

Concluding Remarks

Several studies regarding the prevalence of AFM_1 in industrial and traditional fermented milk were conducted worldwide in the past 20 years, indicating high frequencies of positive samples at low levels of contamination among different industrial and traditional fermented milk products. A decreasing trend in the contamination of fermented milk products was observed over the years, mainly in traditional products. However, AFM_1 contamination in fermented milk at levels higher than the recommended tolerable limits was reported in African and Asian countries. Continuous monitoring and controlling actions from both manufacturers and regulatory bodies are essential to reduce the AFM_1 contamination levels in industrial and traditional fermented milk. Further studies to improve fermentation performance to reduce the AFM_1 contents in contaminated milk and other similar products are recommended.

Conflict of interest statement

The authors declare that there are no conflicts of interest relevant to this study.

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