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Aflatoxin M1 Levels in Cheeses in Türkiye: A Review

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ABSTRACT

Milk and dairy products mostly contain mycotoxins such as aflatoxin M_1, aflatoxin M_2, ochratoxin, cyclopiazonic acid, trichothecene, zearalenone, patulin. Mycotoxins in cheese are produced by certain types of fungi. These either directly contaminate the cheese or feed, or indirectly contaminate the milk used in cheese production. Aflatoxin, causes serious impacts on human and animal health, thus costs the world economy billions of dollars. The first legal regulation regarding aflatoxin M_1 in Turkiye came into force in 1990. Subsequently, various legislations were published in 1997, 2002, 2008, 2011 and 2023. In this study, 60 publications were examined that investigate the presence of aflatoxin M_1 in various cheeses in Turkiye in the last 50 years (1973-2023). In the studies, aflatoxin M_1 was detected in almost all cheeses, and only a few were found in rates exceeding the Turkish Legal Limits. Researchers stated different limits for aflatoxin M_1 in cheese. To minimize the presence of aflatoxin M_1, the following recommendations should be considered. Developing new detection methods, enacting new legal regulations, increasing the frequency of legal inspections, improving the production, transportation and storage conditions of milk and dairy products, improving feed quality, increasing awareness of the health effects of aflatoxin M_1.

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Introduction

Uncontrolled mold growth or some molds are harmful to human health and pose a serious threat to food chains. Molds can multiply in most acidic products (like fruits or juices) and in foods with moderate moisture content (like breads and baked goods) (Jubayer et al., 2021). Molds are found in large quantities in the environment and cause many problems in agriculture, medicine, food, pharmaceutical and cosmetic industries, etc. (Lecellier et al., 2015). The food groups that cause mold multiply are rich in carbohydrates, protein, fat and moisture (Garnier et al., 2017). Molds produce chemicals called mycotoxins, which can be carcinogenic, mutagenic, teratogenic and toxic to humans and animals (Moss, 1990).

Mycotoxins are secondary metabolic products of fungi of *Penicillium*, *Aspergillus*, *Fusarium*, *Rhizoctonia*, *Claviceps* and *Stachybotrys* (Leś et al., 2023). Aflatoxins, zearalenone, deoxynivalenol, fumonisins and ochratoxin A are the main mycotoxins (Abd-Elsalam & Rai, 2019). Aflatoxin, one of the mycotoxins, is mostly multiplied by *Aspergillus parasiticus* and *flavus*. Aflatoxin can occur in dry oily fruits (hazelnuts, peanuts, etc.), dried fruits (dried plums, dried mulberries, etc.), oilseeds, cereals and cereal products, and animal foods (milk, meat, cheese). It causes serious impacts on human and animal health, thus costs the world economy billions of dollars (Shabeer et al., 2022). Aflatoxins were found in 1960 with the association of "Turkey X disease". Since then, it has been understood that low levels of mold metabolites in foods and feeds can cause disease in humans and animals (Leś et al., 2023). Aflatoxins presence in foods and feeds and are toxic metabolites (Garrido et al., 2003). Studies have determined that aflatoxins are seriously carcinogenic (Kimanya et al., 2021; Koshiol et al., 2017; Marchese et al., 2018; Saha Turna & Wu, 2019; Wang & Tang, 2004).

When ruminants consume foods containing aflatoxin B_1 and aflatoxin B_2, these toxins are metabolized and excreted through milk as aflatoxin M_1 and aflatoxin M_2 (Garrido et al., 2003). About 0.3-6.2 % of consumed aflatoxin B_1 is changed to aflatoxin M_1 (AFM1) in the liver (lactating animals) via cytochrome P 450 and excreted into milk (Iqbal et al., 2015; Ketney et al., 2017).

AFM1 can be presence in dairy products due to the binding to milk proteins, especially casein (Vaz et al., 2020). Milk and dairy products mostly contain mycotoxins like AFM1, AFM2, ochratoxin, cyclopiazonic acid, trichothecene, zearalenone, patulin (Oruç, 2014). The International Agency for Research on Cancer (IARC) determines AFM1 as a Group 2B agent (possibly carcinogenic to humans) (IARC, 1993).

Cheese is an ideal substrate for mold growth but a poor substrate for mycotoxin production (Bullerman, 1981). Mold formation in cheese causes problems (about food safety and food quality) and significant economic losses (Kure & Skaar, 2019). There are approximately 4000 types of cheese in World and Turkiye is rich in cheese diversity (Çakmakçı & Salık, 2021). About 200 different types of cheese are produced in Turkiye, excluding traditional cheeses produced in limited areas (Öründü & Tarakçı, 2021). Mycotoxins in cheese are produced by certain types of fungi. These either directly contaminate the cheese or feed, or indirectly contaminate the milk used in cheese production (Hymery et al., 2014). Mycotoxins isolated from cheeses are AFM1, cyclopiazonic acid, isofumigaclavine A, mycophenolic acid, patulin, penitrem A, PR imine, roquefort C, sterigmatocystin, penicillic acid and ochratoxin A (Richard & Arp, 1979; Scott et al., 1977; Sengun et al., 2008).

The levels of mycotoxins reported in cheese are quite low. Some studies indicate that they are not risky for human health (Dobson, 2017). Sengun et al. (2008) stated that cheese does not optimum for toxin production because of low carbohydrate level and has low temperature during ripening.

Legal Regulations Regarding Aflatoxin M_1 Levels in Cheeses in Türkiye

The first legal regulation regarding AFM1 in Turkiye came into force in 1990. Subsequently, various legislations were published in 1997, 2002, 2008, 2011 and 2023. The legislations published regarding AFM1 are shown in chronological order in Table 1.

In the Communiqué on Aflatoxin Control in Turkiye in 1990, aflatoxin M_1 and B_1 were evaluated together and the maximum limit was determined as 0.50 ppb (500 ng/kg) for milk and dairy products (Resmi, 1990). Later, in the Turkish Food Codex Regulation in 1997, AFM1 was evaluated alone for cheese for the first time. In this regulation, the maximum limits are 0.00025 mg/kg (250 ng/kg) for cheese and 0.00005 mg/kg (50 ng/kg) for milk and dairy products (Resmi, 1997). The first limitation of AFM1 for cheese in Turkish Legislation was determined by this regulation.

Afterwards, the Turkish Food Codex Communiqué on Contaminants was published in 2002. In this communiqué, the maximum limit of AFM1 for cheese was evaluated as 0.25 µg/kg (250 ng/kg) (Resmi, 2002). The European Union Commission Regulations began to be cited as a basis for legislation published in 2008 and later. In 2008 and later, no specific limit was specified for cheese; the limits were determined as "raw milk, heat-treated milk and milk used in the production of MBPs (milk-based products)". In the Turkish Food Codex's published in 2008, 2011 and 2023, the maximum AFM1 level for "raw milk, heat-treated milk and milk used in the production of MBPs" was determined as 0.050 µg/kg (50 ng/kg) (Resmi, 2008, 2011, 2023). Over the years, with the development of technology and industry, new methods and devices have been developed for the determination of contaminants. The levels of AFM1 detected in cheeses have decreased and the legal limits have been lowered accordingly. The reasons for this can be listed as follows;

- The lower detection limits of new devices
- More hygienic production of cheeses
- Reducing the risk of contamination
- Improving storage conditions
- More attentive preparation of animal feeds

Studies on Aflatoxin M_1 Levels in Cheeses in Turkiye

In this study, 60 publications investigating the presence of AFM1 in various cheeses in Turkiye in the last 50 years were examined (1973-2023) (Table 2a, 2b). Doctoral and master's theses were excluded from the study. Researchers examined cheeses such as white, tulum, kashar, processed, blue mold, herby, cecil, lor, cream, pickled, surk, plaited, bovine, ovine, caprine, urfa, mihalic, organic, string, halloumi, cokelek, herby lavash, ezine, gruyere and cube. While some researchers examined only one type of cheese regarding AFM1 levels, some of them examined more. Rates exceeding TLL (Turkish Legal Limit) were written as specified by the researchers, and unspecified rates were calculated.

Table 1 Legislations published in Turkiye regarding AFM1

Official Gazette (References)	Legislation Name	Section	Maximum Limits for AFM1
2 May 1990 – 20506 *(Resmi, 1990)*	Communiqué on Aflatoxin Control (KKGM 90/1)	Article 1	0.5 ppb (Aflatoxin B_1+M_1) (Milk And Dairy Products)
16 November 1997 – 23173 *(Resmi, 1997)*	Turkish Food Codex Regulation	Annex-14 Microbial Toxins - Aflatoxin M_1	0.00025 mg/kg (Cheese) 0.00005 mg/kg (Milk And Dairy Products)
23 September 2002 – 24885 *(Resmi, 2002)*	Turkish Food Codex Communiqué on Maximum Limits of Certain Contaminants in Foodstuffs (Communiqué No: 2002/63)	Annex-1 Microbial Toxins - Aflatoxin M_1 (Table 1)	0.25 µg/kg (Cheese)
17 May 2008 – 26879 *(Resmi, 2008)*	Turkish Food Codex Communiqué on Maximum Limits of Contaminants in Foodstuffs (Communiqué No: 2008/26)	Annex-2 Mycotoxins-Aflatoxin M_1 (2.1.5) (2.1.10)	0.050 µg/kg (Raw Milk, Heat-treated Milk and Milk Used in the Production of MBPs); 0.5 µg/kg (Other Foodstuffs (Potentially Risky Foods))
29 December 2011 – 28157 *(Resmi, 2011)*	Turkish Food Codex Regulation on Contaminants	Annex-1 Mycotoxins-Aflatoxin M_1 (2.1.12)	0.050 µg/kg (Raw Milk, Heat-treated Milk and Milk Used in the Production of MBPs)
5 November 2023 – 32360 *(Resmi, 2023)*	Turkish Food Codex Regulation on Contaminants	Annex-1 Mycotoxins-Aflatoxin M_1 (1.1.16)	0.050 µg/kg (Raw Milk, Heat-treated Milk and Milk Used in the Production of MBPs)

Table 2a. Incidence of AFM1 in cheeses in Turkiye (1973-2023)

Cheese Type	AFM1 Occurrence Rates, %	AFM1 Occurrence Rates Exceed TLL, %	References
White	0.00 %	Undefined	(Demirer, 1973)
Tulum	0.00 %		
Kashar	0.00 %		
Processed	0.00 %		
Blue Mold	0.00 %	Undefined	(Demirer, 1974)
Tulum	0.00 %		
Herby	0.00 %		
White	0.00 %	Undefined	(Demirer et al., 1989)
Tulum	0.00 %		
Herby	0.00 %	Undefined	(Kıvanç, 1990)
White	0.00 %		
Kashar	0.00 %	Undefined	(Kıvanç, 1992)
Mixed	45.20 %	1.33 %*	(Dağoğlu et al., 1995)
Kashar	0.00 %	Undefined	(Gürbüz et al., 1999)
Tulum	0.00 %		
White	0.00 %		
White	89.47 %	12.28 %*	(Oruc & Sonal, 2001)
White	65.00 %	Undefined	(Aycicek et al., 2002)
Kashar	60.00 %	32.55 %*	(Günşen & Büyükyörük, 2003)
White	82.00 %	27.00 %*	(Sarimehmetoglu et al., 2004)
Tulum	81.00 %	24.00 %*	
Kashar	85.00 %	34.00 %*	
Processed	79.00 %	25.00 %*	
White	39.13 %	0.00 %*	(Gürses et al., 2004)
Kashar	42.86 %	0.00 %*	
Tulum	63.64 %	0.00 %*	
Cecil	44.44 %	0.00 %*	
Lor	33.33 %	0.00 %*	
White	5.00 %	1.00 %*	(Yaroglu et al., 2005)
Kashar	6.00 %	1.00 %*	
Processed	4.00 %	1.00 %*	
Kashar	56.00 %	4.00 %*	(Çetin et al., 2005)
Herby	86.70 %	80.00 %*	(Tekinsen & Tekinsen, 2005)
White	62.00 %	40.00 %*	
Mixed	90.58 %	8.52 %*	(Aycicek et al., 2005)
Cecil	20.00 %	0.00 %*	(Kamber, 2005)
Kashar	13.30 %	0.00 %*	
Mixed	93.66 %	22.04 %*	(Başkaya et al., 2006)
White	100.00 %	2.00 %*	(Alkan & Gönülalan, 2006)
Mixed	28.21 %	0.00 %*	(Gürbay et al., 2006)
White	95.00 %	20.00 %*	(Kireçci et al., 2007)
Kashar	80.00 %	30.00 %*	
Processed	75.00 %	10.00 %*	
Mixed	71.42 %	38.08 %*	(Yapar et al., 2008)
White	6.25 %	0.00 %*	(Ardic et al., 2008)
Cream	99.00 %	18.00 %*	(Tekinşen & Uçar, 2008)
Kashar	82.60 %	27.30 %*	(Tekinşen & Eken, 2008)
Pickled	82.40 %	26.40 %*	(Ardic et al., 2009)
Surk	60.00 %	13.30 %*	(Aygun et al., 2009)
Plaited	46.67 %	14.44 %*	(Erkan et al., 2009)
White	80.00 %	5.00 %*	(Var & Kabak, 2009)
Kashar	50.00 %	5.00 %*	
White	60.00 %	13.30 %**	(Hampikyan et al., 2010)
Kashar	40.00 %	6.70 %**	
Tulum	55.00 %	10.00 %**	
Bovine	81.82 %	9.09 %*	(Turgay et al., 2010)
Ovine	0.00 %	0.00 %*	
Caprine	77.78 %	0.00 %*	
Mixed	28.00 %	10.00 %*	(Filazi et al., 2010)
White	12.00 %	0.00 %***	(Aksoy et al., 2010)
Kashar	80.00 %	0.00 %***	
White	82.40 %	16.50 %***	(Aydemir Atasever et al., 2010)
Kashar	80.00 %	14.70 %***	
Cecil	19.40 %	00.00 %***	
Cream	84.20 %	6.10 %***	

Table 2b. Incidence of AFM1 in cheeses in Turkiye (1973-2023)

White	7.14%	0.00 %**	(Er et al., 2010)
White	66.70 %	5.60 %*	(Gücükoğlu et al., 2010)
Kashar	76.50 %	29.40 %*	
Tulum	0.00 %	0.00 %*	
Processed	20.00 %	0.00 %*	
String	88.90 %	55.50 %*	
White	70.00 %	0.00 %*	(Ertas et al., 2011)
Kashar	40.00 %	5.00 %*	
Tulum	80.00 %	10.00 %*	
White	28.30 %	10.20 %*	(Kav et al., 2011)
Tulum	60.00 %	0.00 %***	(İşleyici et al., 2011)
White	88.90 %	6.67 %***	(Kocasari et al., 2012)
Urfa	50.00 %	0.00 %**	(Dinçel et al., 2012)
Cecil	0.00 %	0.00 %**	
Mihalic	0.00 %	0.00 %**	
Herby	0.00 %	0.00 %**	
Kashar	0.00 %	0.00 %**	
White	73.50 %	15.69 %*	(Dinçoğlu et al., 2012)
Kashar	57.80 %	19.28 %*	
Blue Mold	0.00 %	Undefined	(Güley et al., 2013)
Organic	43.00 %	7.70 %*	(Tosun & Ayyildiz, 2013)
Kashar	97.96 %	10.88 %***	(Gul & Dervisoglu, 2014)
White	20.00 %	0.00 %*	(Temamogullari & Kanici, 2014)
Kashar	50.00 %	2.50 %***	(Bakirdere et al., 2014)
Tulum	18.75 %	0.00 %***	
String	13.64 %	0.00 %***	
Cream	38.10 %	0.00 %***	
White	53.73 %	7.46 %***	
Halloumi	28.80 %	0.00 %***	(Öztürk et al., 2014)
(Industrial)	21.70 %	0.00 %***	
Halloumi (Traditional)			
Blue Mold	41.70 %	0.00 %*	(Koluaçık et al., 2015)
White	92.60 %	0.00 %*	(Sarıca et al., 2015)
Blue Mold	52.00 %	17.00 %*	(Özgören & Seçkin, 2016)
White	79.39 %	0.96 %***	(Yeşil et al., 2019)
Cecil	100.00 %	0.00 %***	
Cokelek	66.67 %	0.00 %***	
Cream	100.00 %	0.00 %***	
Lor	83.33 %	0.00 %***	
Herby	62.79 %	3.70 %***	
Herby Lavash	38.46 %	0.00 %***	
White	40.00 %	0.00 %**	(Öztürk Yilmaz & Altinci, 2019)
Kashar	65.40 %	0.00 %**	
White	60.00 %	0.00 %**	(Acaroz, 2019)
Tulum	67.50 %	0.00 %**	
Ezine	50.00 %	0.00 %**	(Eker et al., 2019)
Blue Mold	16.00 %	0.00 %***	(Aksoy & Sezer, 2019)
Kashar	36.00 %	0.00 %***	
Gruyere	68.00 %	0.00 %***	
Cube	100.00 %	1.11 %**	(Ağaoğlu et al., 2020)
White	100.00 %	100.00 %**	(Demir et al., 2021)
Processed	100.00 %	1.20 %**	(Mortaş et al., 2022)
White	35.71 %	2.38 %***	(Ergin et al., 2023)
Tulum	45.23 %	0.00 %***	

AFM1 Occurrence Rates: Shows how many of 100 samples contain AFM1 (as a percentage); TLL: Turkish Legal Limit; * TLL: 250 ng/kg, 0.25 µg/kg, 0.00025 mg/kg, 250 ppt, 0.25 ppb, 0.00025 ppm; ** TLL: 50 ng/kg, 0.05 µg/kg, 0.00005 mg/kg, 500 ppt, 0.050 ppb, 0.00005 ppm; *** TLL: 500 ng/kg, 0.50 µg/kg, 0.0005 mg/kg, 500 ppt, 0.50 ppb, 0.0005 ppm

In studies until 1995, AFM1 could not be detected in cheeses, so rates exceeding the TLL were not mentioned. In 1995, Dağoğlu et al. (1995) indicated the AFM1 in cheese as 45.20 % and the rate of exceeding TLL as 1.33 % (out of 100 samples). In almost all studies in 2001 and later, AFM1 was detected in varying rates in cheeses.

In the communiqués published in 1997 and 2002, the term "cheese" was clearly stated and the legal limit was 250 ng/kg. In the legal regulations of the following years (2008, 2011 and 2023), the term "cheese" was not clearly stated, and instead the maximum AFM1 limit for "raw milk, heat-treated milk and milk used in the production of MBPs" was mentioned as 50 ng/kg. In the communiqués published in 2008, the maximum AFM1 limit for "potentially risky foods" (other foodstuffs) was mentioned as 500 ng/kg.

In many studies, researchers have noted both the presence of AFM1 and rates exceeding TLL in cheese. Although there are differences according to the years in which the legal regulations were published;

- Some studies cited the expression "cheese" as reference (250 ng/kg),
- Some studies cited the expression "raw milk, heat-treated milk and milk used in the production of MBPs" as reference (50 ng/kg),
- Some studies cited the expression "potentially risky foods" as reference (500 ng/kg).

TLLs cited by researchers are indicated with * in Table 2.

Results

Aflatoxins are toxic metabolites for human and animals. It is necessary to determine the maximum limits of presence in foods for these metabolites that have been proven to be toxic to humans and animals. So, legal limits have been determined in many countries and various international committees. Many regulations have been enacted for AFM1 in Turkiye over the years and researchers have referred the limits in these regulations in studies.

In this review, the studies on AFM1 in cheese was examined and it was noticed that researchers cited different limits. In the studies, AFM1 was detected in almost all cheeses, and only a few were found in rates exceeding the TLL.

Discussion

Oruç (2003) examined the levels of Aflatoxin M1 (AFM1) in milk and dairy products in Turkiye. The study stated that the rate of AFM1 in milk and dairy products in Turkiye is high and that milk and milk products may contain AFM1 at levels that could threaten public health. This review is parallel to the mentioned study, includes more current studies and is only specific to "cheese".

Studies published in Türkiye are mostly related to "milk and dairy products". There are many studies on topics such as the possible risks of aflatoxins in milk and dairy products (Ay & Şanlı, 2018; Özdemir & Demirer, 2021; Şimşek & Ağaoğlu, 2023), residue risks (Çoşkun & Şanlı, 2016; İnce & Karatekeli, 2020; Mortaş et al., 2022), the presence of peptides (Ay & Şanlı, 2018; Özdemir et al., 2021), the presence of microplastics (Halıcı Demir et al., 2024) and the presence of some pathogens (Ekici et al., 2004; Mohamed & Alçay, 2020; Sipahi & Çelik Doğan, 2023). The wide variety of cheeses in Turkiye and the high level of traditional production as well as industrial production have necessitated the examination of cheese separately from other dairy products.

In studies published between 1973 and 1992, researchers did not detect AFM1 in cheese varieties. The reasons for this may be technological deficiencies or high detection limits of the devices. The existence of AFM1 was mentioned in almost all of the studies published in subsequent years. It is thought that the methods used by researchers affect the results both qualitatively and quantitatively. In the studies, researchers generally preferred Capillary Electrophoresis, HPLC, ELISA, GC and GC-MS methods. The development of these methods has made the analyses more sensitive over the years. Until 2010, researchers cited 250 ng/kg as the reference amount exceeding the TLL. As explained below, different limits were cited as reference even in studies conducted in the same year. For example, in 2010, it was observed that researchers cited 250 ng/kg (Filazi et al., 2010; Gücükoğlu et al., 2010; Turgay et al., 2010), 50 ng/kg (Er et al., 2010; Hampikyan et al., 2010) and 500 ng/kg as reference values (Aydemir Atasever et al., 2010).

Conclusion

In the latest legal regulations, no maximum legal limit has been specified for AFM1 in "cheese". It should be defined with the new regulations. Developed countries are making special efforts to reduce AFM1 in milk and dairy products. To minimize the presence of AFM1, the following recommendations should be considered.

- Developing new detection methods
- Enacting new legal regulations
- Increasing the frequency of legal inspections
- Improving the production, transportation and storage conditions of milk and dairy products
- Improving feed quality

Increasing awareness of the health effects of AFM1

Declarations

Author Contribution Statement

B.O: Data collection, writing the original draft, investigation, review and editing.

Conflict of Interest

"The author declares no conflict of interest."

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