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Investigation of aflatoxin M1 levels of fresh white cheeses offered for sale in Elazig, Kovancilar, Bingol, and Tunceli, in Turkey

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Abstract

In this study, in fresh white cheeses collected from Elazig, Kovancilar, Bingol, and Tunceli in Turkey were examined presence and residue levels of Aflatoxin M1 (AFM1). For this purpose, twenty-four white cheese samples were collected from each city, and a total of 96 white cheese samples were collected periodically from March to August in 2020. Aflatoxin analysis was performed by ELISA method. As a result of the analysis, the incidence of AFM1 in the fresh white cheese samples collected from Elazig, Kovancilar, Bingol, and Tunceli ranged from 0.71 to 4.50 µg/kg, from 1.80 to 4.33 µg/kg, from 0.12 to 3.96 µg/kg, and from 1.35 to 3.51, respectively. The AFM1 level in the analyzed cheese samples was found higher than 0.05 µg/kg, which is the limit value for AFM1 stated in the Turkish Food Codex. As a result, AFM1 levels of fresh white cheeses produced at different sales points in different provinces pose a potential risk for consumers in terms of public health, farmers/producers/employees in feed and cheese production companies need to be informed about the aflatoxin and aflatoxicosis and terms of hygiene and sanitation rules to be followed during production, and also the continuous monitoring of the aflatoxin level must be applied by the Turkish public health authorities.

Keywords: aflatoxin M1, ELISA, fresh white cheese, public health

1. INTRODUCTION

Cheese is a milk product with a wide variety of types and has an important place among dairy products (Erkan, 2018). White cheese is one of the most produced cheese variety in Turkey. Its commercial production is centered around the Thrace Region, Marmara Region, Aegean Region, and Konya, which have high sheep milk production and are developed in terms of storage and transportation facilities.

Aflatoxins are a class of toxic substances that are classified under mycotoxins. They are mainly produced by molds, including *A. flavus*, *A. parasiticus*, and *A. nomius*. Aflatoxin formation depends on the type of food on which the molds can grow in addition to the temperature and humidity conditions prevailing during growing, harvesting, and storage of the products. Aflatoxins B1, B2, G1, G2, and their metabolites, M1 and M2 are the most common, of which B1 and M1 are the most commonly encountered aflatoxins in foods. Aflatoxin M1 (AFM1) is formed as a hydroxylated metabolite of aflatoxin B1 (AFB1) in the liver of animals consuming feed contaminated with AFB1 and passes through the mammary glands into milk. It is known that AFM1 is a class one carcinogen (Bodbodak et al., 2018; Xiong et al., 2020; Xiong et al., 2021). The maximum limit for AFM1 concentration in milk and dairy-based products is 0.05 µg/kg as per Turkish Food Codex Regulation on Contaminants (TGK, 2011).

Milk and dairy products are major sources of aflatoxin contamination among food products. This results in potential exposure to AFM1. However, it is noteworthy that storage and processing procedures do not cause a decrease in the amount of Aflatoxin M1 (Unusan, 2006). Additionally, AFM1

is relatively stable in raw and processed dairy products and is not effected by pasteurization, ultra-high temperature sterilization, and/or conversion of milk to cheese (Yaroglu et al., 2005; Unusan, 2006).

There are many important studies with AFM1 in milk and dairy products in recent years. (Campagnollo et al., 2016; Ketney et al., 2017; Bodbodak et al., 2018; Jaiswal et al., 2018; Xiong et al., 2018a; Xiong et al., 2018b; Pena-Rodas et al., 2018; Guo et al., 2019; Sevim, 2019; Fakhria & Khaneghah, 2020; Gonçalves et al., 2020. Xiong et al., 2020; Xiong et al., 2021).

In view of the strong carcinogenicity of AFM1 and the susceptibility of milk to AFM1 contamination, as well as few reports on the contamination of white cheese in Turkey in recent years. This study aimed to investigate the levels of AFM1 in fresh white cheese collected from Elazig, Kovancilar, Bingol, and Tunceli, the important areas of white cheese consumption in Turkey.

2. MATERIALS AND METHODS

2.1. Materials

Fresh white cheese samples were collected from Elazig, Kovancilar, Bingol, and Tunceli between March and August 2020. Twenty-four white cheese samples were collected from each city, and a total of 96 white cheese samples were collected. The samples were collected randomly in sterile sample bags and delivered using cold chain to the laboratory of Department of Food Hygiene and Technology, Firat University Faculty of Veterinary Medicine. Additionally, the cheese samples were refrigerated until analysis was performed.

2.2. Methods

AFM1 levels in cheese samples were determined by enzyme-linked immunosorbent assay (ELISA) using Agra Quant-Romer Labs (KMTKS-ROM-MT-03) Aflatoxin M1 test kit (Agra Quant, 2016).

2.2.1. Preparation of Cheese Samples

For analysis, 200 g of the cheese samples were homogenized followed by transferring 2 g of the sample to a 50 mL falcon tube. Then, 40 mL of dichloromethane was added to the sample tubes, which were subsequently placed on an orbital shaker and agitated at 260 rpm for 30 minutes. After agitation, 5 mL of this mixture was transferred to glass tubes and evaporated at 60°C. The evaporation product was dissolved in a mixture of 0.5 mL phosphate buffered saline (PBS) + 0.5 mL methanol + 1 mL hexane. It was then transferred from the methanol/water phase (lower phase) to an eppendorf tube of 0.4 mL and mixed with 0.6 mL of sample diluent. Subsequently, this mixture was used for ELISA.

2.2.2. Test Procedure

For ELISA, 200 µL conjugate and 100 µL diluted sample extract/standard were added to green-rimmed dilution wells and mixed three times by pipetting. Subsequently, 100 µL of this mixture was transferred to the test well. This was followed by incubation for 60 minutes at room temperature in dark conditions. The wells (strips) were washed five times with diluted wash buffer using a washer (Biotek. ELx50, U.S.A.). After washing, the wells (strips) were dried upside down on paper towels. Once dry, 100 µL of substrate was added to each well followed by incubation for 20 minutes at room temperature in dark conditions. Post incubation, 100 µL of stop solution was added. The absorbance measurement was performed at a wavelength of 450 nm (630 nm reference) using an ELISA reader (Biotek. ELx800, U.S.A.). without retention after color change from blue to green. The absorbance (optical density) values obtained were automatically calculated in KJ Junior program using a calibration curve created by transferring them to Romer Labs Excel calculation table. In the AFM1 measurements, 25, 50, 100, 200, and 500 ppt of standards were used to obtain a calibration curve. The limit of detection of the test kit used in cheese is 128 ppt, the range of quantitation is 200-4000 ppt, and the recovery is 61-69%.

2.3. Statistical Analysis

The descriptive statistics of the cheese samples analyzed in the study and the relationships between the values were made using the SPSS 22

(IBM SPSS, IBM Corporation, USA) package program. Analysis results were given as mean ± standard deviation (Ozdamar K, 1999).

3. RESULTS

The AFM1 results obtained in this study are given in Table 1 and distribution of AFM1 levels of fresh white cheese samples according to the maximum limit value of Turkish Food Codex (TGK, 2011) is given in Table 2

It was found that the values for AFM1 concentration in all cheese samples from the four regions were higher than those reported in the TGK (2011) (Tables 1 and 2). According to the analysis results, the incidence of AFM1 in the fresh white cheese samples collected from Elazig, Kovancilar,, Bingol, and Tunceli ranged from 0.71 to 4.50 µg/kg, from 1.80 to 4.33 µg/kg, from 0.12 to 3.96 µg/kg, and from 1.35 to 3.51, respectively. Mean values for AFM1 were 2.65 ± 1.11 µg/kg in Elazig, 3.27 ± 0.83 µg/kg in Kovancilar,, 1.95 ± 1.19 µg/kg in Bingol, and 2.55 ± 0.74 µg/kg in Tunceli (Table 1).

Table 1. Aflatoxin M1 levels of fresh white cheese samples

Place taken of samples	Number of samples	Mean ± Standart deviation	AFM1 levels (min-max)
Elazig	24	2.65 ± 1.11	0.71 - 4.50
Kovancilar	24	3.27 ± 0.83	1.80 - 4.33
Bingol	24	1.95 ± 1.19	0.12 - 3.96
Tunceli	24	2.55 ± 0.74	1.35 - 3.51

AFM1 level of the white cheese samples sold in Elazig city, 0.5-1.0 µg/kg in 2 sample, 1.0-2.0 µg/kg in 6 sample, 2.0-3.0 µg/kg in 7 sample, 3.0-4.0 µg/kg in 6 sample, and > 4.0 µg/kg in 3 sample was detected. Samples sold in Kovancilar, region, 1.0-2.0 µg/kg in 2 sample, 2.0-3.0 µg/kg in 6 sample, 3.0-4.0 µg/kg in 10 sample, and > 4.0 µg/kg in 6 sample was detected. Samples sold in Bingol city, 0.5-1.0 µg/kg in 7 sample, 1.0-2.0 µg/kg in 2 sample, 2.0-3.0 µg/kg in 11 sample, 3.0-4.0 µg/kg in 4 sample was detected. Samples sold in Tunceli city, 1.0-2.0 µg/kg in 9 sample, 2.0-3.0 µg/kg in 6 sample, 3.0-4.0 µg/kg in 9 sample was detected (Table 2).

Table 2. Distribution of AFM1 levels of fresh white cheese samples (µg/kg) (n: 24).

Place taken of samples	AFM1 levels (µg/kg)									
	0.5 - 1.0		1.0 - 2.0		2.0 - 3.0		3.0 - 4.0		> 4.0	
	n	n(%)	n	n(%)	n	n(%)	n	n(%)	n	n(%)
Elazig	2	8.33	6	25	7	29.17	6	25	3	12.5
Kovancilar	-	-	2	8.33	6	25	10	41.67	6	25
Bingol	7	29.17	2	8.33	11	45.83	4	16.67	-	-
Tunceli	-	-	9	37.5	6	25	9	37.5	-	-

4. DISCUSSION AND CONCLUSION

In this study, in fresh white cheeses collected from Elazig, Kovancilar, Bingol, and Tunceli were examined presence and residue levels of Aflatoxin M1. For this purpose, twenty-four white cheese samples were collected from each city, and a total of 96 white cheese samples were collected.

AFM1 levels determined in this study are similar to those obtained in studies on white cheese (Özmenteşe, 2002; Alkan and Gonulalan, 2006) and tulum cheese (Erkan et al., 2018). In these studies, with white cheese, the average Aflatoxin M1 level was found at the level of 16-713 ng/kg by Özmentese (2002), while Alkan and Gonulalan (2006) found it at the level of 30-1237 ng/kg. Erkan et al. (2018) found Aflatoxin M1 level to be 64-432 ng/kg in their study on tulum cheese. Additionally, it was observed that AFM1 levels detected in this study were higher than those obtained in other studies on white cheese (Table 3).

AFM1 levels in cheese are typically higher than milk samples owing to the high affinity of AFM1 to casein (Galvano et al., 1996; Battacone et al., 2005; Costamagna et al., 2019). Hence, the process steps should be controlled for detecting the stage at which aflatoxin contamination occurs during the cheese production process and for preventing this contamination (Wisemann et al., 1983).

Table 3. AFM1 levels determined in different types of cheese researches in Turkey (ng/kg) (Agaoglu et al., 2020)

Types of cheese	Province	Sample Number	n-n(%)	>250	>500	AFM1 levels (min-max)	Detection Method	References
Van herby and pickled white cheese	Van	50	0					Kivanc (1990)
White Cheese	İstanbul	75	36(45.2)		1	60-510	ELISA	Dagoglu et al. (1995)
White Cheese	Konya	240	0					Gürbüz et al. (1999)
White, Kashar, soft cheese	Ankara	150	0					Kardes (2000)
White-pickled cheese and Kashar cheese	Van	90					TLC	Bakirci. (2001)
Fatty white cheese	Bursa	57	(89.7)	7(12.28)	1	40-810		Oruc and Sonal (2001)
White Cheese	Marmara	110	101(91.8)	6		10-2000		Seyrek (2001)
White Cheese	İstanbul	186	121(65)	35(19)		40-4890		Aycicek et al. (2002)
White Cheese								Ozkaya (2002)
White Cheese	İstanbul	15	15(100)	-13.3		16-713	HPLC	Özmenteşe (2002)
White Cheese								Yaroglu (2002)
Fresh kashar cheese	Bursa	125	86(68.8)	19(22.09)	9(10.46)	10-740	ELISA	Gunsen and Buyukyoruk (2003)
Kashar Cheese	Ankara	25	14(56)	1		10-400	HPLC	Cetin (2004)
White, kashar, tulum, processed cheeses	Ankara	400	327(81.7)	110(27.5)			ELISA	Sarımehmetoglu et al. (2004)
White, kashar, tulum, lor and civil cheeses	Erzurum	63	28(44.4)			7-202	ELISA	Gurses et al. (2004)
Civil Cheese	Erzurum	50	47(94)	3(6)			ELISA	Baskaya (2004)
White and kashar cheeses	Ankara	196	177	19			ELISA	Aycicek et al. (2005)
Civil cheese								Baskaya and Atasever (2005)
White, kashar, processed cheeses	İstanbul	363	80 (22.04)				ELISA	Baskaya et al. (2006)
White, kashar, processed cheeses	Bursa. Bilecik. Eskisehir. Ankara. Izmir. Istanbul. Manisa. Usak. Kırıkkale. Cankırı. Kastamonu. Zonguldak. Bartin.	600	30(5)	6(1)		100-800	ELISA	Yaroglu et al. (2005)

	Kutahya. Balikesir and Canakkale							
Van Herby and white pickle cheeses	Van	110	83	68		100-7260	AflaTest	Tekinsen and Tekinsen (2005)
Cecil Cheese and Kars Kashar Cheese	Kars	60	10			51-115	ELISA	Kamber (2005)
Milk and dairy products	Konya	150	123	47		52-860	ELISA	Ozturan (2005)
White Cheese	Amasya	50	50(100)	1(2)		30-1237	ELISA	Alkan and Gonulalan (2006)
White Cheese. Kashar, tulum, curd cheeses	Aydın	25	25(100)	1(4)		40-250	HPLC	Kok (2006)
Some cheese types	Ankara	39	11(28.21)			78.2-188.4	ELISA	Gurbay et al. (2006)
White, kashar, processed cheeses	Sarıkamıs-Kars	60	48	12			ELISA	Kirecci et al. (2007)
Kashar cheese	Istanbul. Izmir. Konya. Tekirdag. Edirne	132	109(82.6)	36(27.3)		50-690	ELISA	Tekinsen and Eken (2008)
Cream cheese	Istanbul. Izmir. Kayseri. Konya. Tekirdag	100	(99.0)		(18.0)	0-4100	ELISA	Tekinsen and Ucar (2008)
Old kashar cheese, fresh kashar cheese, civil cheese, white cheese, and Gravier cheese	Different Province	105	17	7(28)		51-400	TFC	Yapar et al. (2008)
Urfa Cheese	Sanlıurfa	64	4(6.25)			51.1-99.6	ELISA	Ardıc et al. (2008)
Surk cheese	Southern part of Turkey	120	72(60)	16(13.3)		16-1.043	ELISA	Aygun et al.. 2009
White brined cheese	Erzurum	193	159(82.4)	60		52-860	ELISA	Ardıc et al. (2009)
Diyarbakir Orgu (Knitted) cheese	Diyarbakır	90	42(46.67)	13(14.4)			ELISA	Erkan et al. (2009)
White. kashar and tulum cheeses	İstanbul	80	41(51.3)			52-2520	ELISA	Hampikyan et al. (2010)
Kahramanmaras Cheese	Maras	46	32(69.6)			60-1200	HPLC	Turgay et al. (2010)
Semi-hard cheese	Erzurum	304	216(71.1)	63(20.7)	30(9.9)	51-860	ELISA	Atasever et al. (2010)
White. kashar.Tulum, processed and string(dil) cheeses	Erzincan	64	31	11				Gucukoglu et al. (2010)
White Cheese	Ankara	70	5			0-240	ELISA	Demirhan et al. (2010)
White and kashar cheeses	Samsun	50	25(50)			19.6-41.9	ELISA	Aksoy et al. (2010)
Cheeses produced by dairy ewe's	Sanlıurfa	50	14(28)	5(10)		20-2000	TLC	Filazi et al. (2010)

White, kashar and tulum cheeses	Kayseri	60	38(63)	3		12-378	ELISA	Ertas et al. (2011)
Traditional cheeses	Ege reg.	200	66	8		0.24-837.5	ELISA	Eroglu (2011)
White-brined Urfa cheese	The southeast of Turkey	127	36(28.3)	13 (10.2%)		70.61-770.97	ELISA	Kav et al. (2011)
White and some cheese types	İzmit	185	123(66.5)	32	10	12.3-760.4		Dincoglu et al (2012)
Some cheese types	Burdur	45	40(88.9)	7(15.6)	3(6.7)	55-600	ELISA	Kocasarı et al. (2012)
Kashar and Cream cheeses	Aydin and Izmir							Kogustun (2012)
Civil. Mihalıc. Otlu cheese and Kars cheddar	Urfa. Erzurum. Van. Kars	100	10				HPLC	Dinçel et al. (2012)
Kashar cheese	Karadeniz reg	147	144(97.96)		16(11.1)	15-3774	HPLC	Gul and Dervisoglu (2014)
Halloumi cheese	Kıbrıs	128	(21.7)			0.00-16.6	ELISA	Ozturk et al. (2014)
White pickled cheese	Sanlıurfa	50	10 (20)			103.2	ELISA	Temamogulları (2014)
White, kasar, Tulum, dil, and cream cheese	Kocaeli. Sakarya. Duzce	166	70		5	50-2100	ELISA	Bakırdere et al. (2014)
White Cheese	Ankara	27	25(92.6)			7.3-84.4	HPLC-FLD	Sarica et al. (2015)
Kup cheese	Ankara. Yozgat. Nevsehir	60	25(41.7)		5	16-136	HPLC	Koluacık et al. (2015)
Mouldy cheese	Erzurum. Mersin. Konya. Kayseri.Isparta	100	52	19		10.6-702	ELISA	Ozgoren and Seckin (2016)
Tulum	Elazıg	100	100			64-432	ELISA	Erkan et al. (2018)
Moldy chechil, kashar and gruyere cheese	Kars	150	60(40)			0-230.10	ELISA	Aksoy et al. (2019)
Diyarbakir Orgu (Knitted) cheese	Diyarbakır	184	158(85.87)	32(17.39)		50-800	ELISA	Yeşil et al. (2019)
Cube cheeses	Sivas	90				2.16-53.94	ELISA	Agaoglu et al. (2020)

n: Sample number

There are a large variety of cheese types in our country, and hence many studies on detection of AFM1 in cheeses are present. These studies have been performed on Cheddar, Tulum, Cream, Civil, Plaited, Cube, Gruyere, Blue Mold Cecil, Herby, Sürk, Melted, Urfa, Curd, Dil, Mihaliç, and Hellim cheeses (Table 3). The AFM1 levels determined in this study were higher than those obtained in the above-mentioned studies.

Some researchers have reported no presence (no detected) of AFM1 in their studies on white, Kashar, Tulum, and Cheddar cheese samples (Demirer, 1973; Kivanc, 1990; Gürbüz et al., 1999; Kardes, 2000; Kirecci et al., 2007; Dinçel et al., 2012).

There are many studies on AFM1 levels in cheese worldwide. Although majority of these studies have been conducted in Iran. there are many studies related to AFM1 concentration conducted in Greece, Italy, India, Japan, Syria, Kazakhstan, Spain, Kuwait, El Salvador, Ethiopia, Serbia, Brazil, Saudi Arabia, Qatar, China, and Lebanon. In this studies, the levels of AFM1 were determined in different types of cheeses between 0.6-6920 ng/kg (Table 4).

The Turkish Food Codex (TGK, 2011) and the European Union (EC, 2010) have specified the maximum limit of AFM1 levels permitted for consumption in milk and milk-based products as 0.05 μg/kg (50 ng/kg). The evaluation could not be made since there is no limit value regarding the AFM1 level in cheese in the Turkish Food Codex (TGK, 2011). When the research findings are examined all of the analyzed fresh white cheeses did not comply with the EU reported limit value in terms of AFM1 residue (EC, 2010). The evaluation could not be made since there is no limit value regarding the AFM1 level in cheese in the Turkish Food Codex (TGK, 2011).

As seen above, the levels of AFM1 contamination in cheese vary between studies. This variation may occur due to different factors effecting the degree of contamination in milk, including the amount of AFM1 contaminated milk to be processed into cheese, differences in cheese production technology, cheese type, fermentation degree, salting process, cheese production, ripening and storage conditions, cheese ripening duration, geographical differences, widespread periods of intensive breeding, seasonal changes, animal feeds preserved under unfavorable conditions, and different analysis methods (Blanco, 1988; Barrios et al., 1996; Galvano et al., 1996; Pittet. 1998; Battacone et al., 2005; Kamkar et al. 2008; Fallah, 2010; Tavakoli et al., 2012; Bulca and Bircan, 2013; Ayyash et al., 2014; Erkan et al., 2018).

In this study, it was found that the values for AFM1 concentration in all cheese samples from the four regions were higher than those reported in the TGK (2011).

The results of the analysis reveal that AFM1 levels in fresh white cheese can pose a potential health hazard to consumers. As a precaution, it is necessary to store raw milk and cheese in appropriate conditions (humidity, temperature, etc.), pay attention to production hygiene during cheese production, use healthy raw materials for feed production and store animal feed in appropriate conditions, and to raise awareness among cheese and feed producers. In addition, farmers/producers/employees in feed and cheese production companies need to be informed about the aflatoxin and aflatoxicosis and terms of hygiene and sanitation rules to be followed during production, and also the continuous monitoring of the aflatoxin level must be applied by the Turkish public health authorities. Additionally, it is important to increasing awareness regarding this issue among consumers for preventing risks associated with it.

Table 4. The level of AFM1 in cheeses from different countries researches (ng/kg) (Agaoglu et al., 2020)

Country	Types of Cheeses	n	n-n(%)	> 50 ng/l*	AFM1 Levels (min-max)	Detection Method	References
Southern Spain	Fresh, semicured or semiripened, ripened cheeses	35	16(44.7)		20-200	HPLC	Barrios et al. (1996)
Brazil	Minas cheese	75	56(74.7)	20(26.7)	20-692	HPLC	Prado et al. (2000)
Libya	Fresh White, soft cheese	20	15(75)		110-520	HPLC	Elgerbi et al. (2004)
Italy	Hard, cooked. long matured cheeses					LC-MS	Cavaliere et al. (2006)
Iranian	White -Manchego type cheeses					HPLC	Kamkar et al. (2008)
Italy	Unripened, hard, long term ripened cheeses	265	44(16.6)		50-250	ELISA	Montagna et al. (2008)
Brazil	Minas cheese	88	40(46.4)	2		HPLC	Prado et al. (2008)
Sardinia (Italy)	Goat Cheese	41	4(9.8)		79.5-389	ELISA	Virdis et al.. 2008
Kuwait	White cheese	40	32(80)	13	23.8-452	ELISA	Dashti et al. (2009)
China	Cheese	4	4(100)			ELISA	Pei et al. (2009)
Iranian	White and cream cheese	210	93(80.1)	(24.2)	52.1-785.4	ELISA	Fallah et al. (2009)
Iranian	White cheese	72	59 (81.9%)		30-1200	TLC	Fallah et al. (2010)
Iranian	Creamy-feta cheese	80			21.96-43.31	ELISA	Mohamadi et al. (2010)
Egypt	Traditional cheeses	150	50	50	51.6-182	ELISA	Amer and İbrahim (2010)
Italy	Soft fresh Italian cheese				30-250	HPLC	Cattaneo et al. (2011)
Iranian	White cheese	50	30(60)	3(6)	40.9-374	ELISA	Tavakoli et al. (2012)
Saudi Arabia	White, creamy, Kashar cheese	261	227			ELISA	Ashraf (2012)
Lebanon	Dairy, farms and imported cheeses	111	(67.56)			ELISA	Elkak et al. (2012)
Iranian	White and Lighvan cheese	82	39	10	70.5-309	ELISA	Mohajeri et al. (2013)
Brazil	Parmesan cheese	90	18(60)	8(26.7)		HPLC-FLD	Trombete et al. (2014)
Iranian	Cheese	80	69(86.3)	11(13.8)	14.3-572.1	ELISA	Rahimi (2014)
Lebanese	Akkawi. halloum. karishe.shanklish cheeses	260		105 (20)		ELISA	Hassan and Kassaify (2014)
Costa Rica	Fresh cheese	70	49	13	31-276	HPLC	Chavarría et al. (2015)
Serbia	White and hard cheese	74				ELISA	Tomasevic et al. (2015)
Serbia	White and hard cheese	54				UHPLC-MS/MS	Skrbic et al. (2015)
Iranian	White cheese	10	6(60)		5.8-21.2	ELISA	Sohrabi and Gharahkoli (2016)
Iranian	traditional cheese	40	(65.5)	4		ELISA/HPLC	Bahrami et al. (2016)
Baghdad	White and soft cheese	40	15(53.85)	10		ELISA/HPLC	Al Mossawei et al. (2016)
Italy	Parmesan cheese				275	HPLC	Pietri et al. (2016)
Egypt	Processed cheese and Domiati cheese	30	13	2	12.5-74.23	ELISA	Tahoun et al. (2017)
Qatar	Mozzarella, Edam, Cheddar, cream and Moshalal cheese	46	39(85)			ELISA/HPLC	Hassan et al. (2018)
Lebanon	Halloum, Akkawi, Double Cream and Bulgarian&Chanklish cheese	72	(58.3)	41.7	85-343	HPLC	Daou et al. (2020)
El Salvador	Hard white cheeses	46	39 (84.8)		217.1	ELISA/HPLC	Pena-Rodas et al. (2020)
Ethiopia	Cottage cheese	82	82(100)		800-5580	ELISA	Tadessea et al. (2020)

n: Sample number; n1: Positive sample; n2: Example exceeding the limit; *Limit value: 50 ng/L: EC (2010).

Conflicts of interest

There is no conflicts of interest in this study. A part of this study was published in 3rd International New York Conference on Evolving Trends in Interdisciplinary Research & Practices, November 13-15. 2020, Manhattan, New York City

Declaration of competing interest

The authors declare that no conflict of interests in this study. Manuscript is read and approved by all authors

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