



## Presence of Aflatoxin M<sub>1</sub> in Cube Cheeses Produced in Sivas Region

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ARTICLE INFO	ABSTRACT
<p><i>Research Article</i></p> <p>Received : 09/04/2019 Accepted : 26/02/2020</p> <p><b>Keywords:</b> Cube cheese Aflatoxin M<sub>1</sub> Residue ELISA Sivas</p>	<p>In this study, the presence and residue levels of aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) were investigated in traditionally produced cube cheeses in Sivas and its region. For this purpose, 90 pieces of cube cheese were used as a material. Cheese samples were collected periodically in November and December 2016 from central sales location and surrounding villages. ELISA technique was applied in aflatoxin analyses. According to the analysis results, AFM<sub>1</sub> was detected in total of the cube cheeses. The lowest level of AFM<sub>1</sub> was found to be 2.16 ng/kg, the highest was 53.94 ng/kg and the mean was 6.36±0.87 ng/kg. AFM<sub>1</sub> level was determined one cheese sample above to the limit set by European Union.</p>

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### Introduction

Aflatoxins; mainly *A. flavus* and *A. parvicoccus*, including some types of *Aspergillus*, *Penicillium* and *Rhizopus* are produced as secondary metabolites. The clinical picture of aflatoxins in humans and animals is defined as an 'aflatoxicosis' (Sweeney and Dobson, 1998; Ünlütürk, 1998).

According to their color under ultraviolet (UV); aflatoxin B<sub>1</sub> (AFB<sub>1</sub>), B<sub>2</sub>, G<sub>1</sub>, G<sub>2</sub> and M<sub>1</sub>, M<sub>2</sub> consists of six main compounds of Aflatoxins. AFM<sub>1</sub> and M<sub>2</sub>, AFB<sub>1</sub> and B<sub>2</sub> known as milk toxins are milk-excreted derivatives. The most toxic of aflatoxins is AFB<sub>1</sub>. Carcinogenic effect of aflatoxin M<sub>1</sub> is 10 times lower than AFB<sub>1</sub> (Kaya, 2001; Agag, 2004).

Aflatoxins are compounds that have a toxic effect on human and all animal species. In addition to carcinogenic, mutagenic, teratogenic, hepatotoxic and immunosuppressive properties of aflatoxins, they have been reported to be effective in the development of kidney damage and various of organ tumours (Gerbers and Caselman, 1995; Kaya, 2001).

AFB<sub>1</sub> is defined as 'Class 1 carcinogen' and AFM<sub>1</sub> is defined as 'Class 2B probable in human carcinogens' by

International Agency for Research on Cancer (IARC) (Rothschild, 1992; IARC, 1993). AFM<sub>1</sub> was included in the Class 1 list in the classification made in 2002 (IARC, 2002).

Aflatoxins are highly resistant to heat treatment. It has been reported that aflatoxin M<sub>1</sub> maintains its stability in pasteurization and processing of milk into various products, but is completely disintegrated at 300°C and higher (Galvano et al., 1996).

Due to the negative effects on human and animal health, legal regulation has been introduced for aflatoxins in many countries. The Commission of the European Union (EU) (EC, 2010) reported the maximum AFM<sub>1</sub> level in milk and dairy products as 50 ng/kg. Turkish Food Codex Regulation on Contaminants (TGK, 2011) was determined the maximum limit of aflatoxin M<sub>1</sub> in raw milk, heat-treated milk and milk-based products as 0.050 µg / kg. This level is reported as 0.025 µg/kg for infant formulas and continuation formulas (including infant milk and continuation milk).

Aflatoxin residue in milk is shaped by the livestock consumption of feeds contaminated with AFB<sub>1</sub> and AFB<sub>2</sub>. AFB<sub>1</sub> and AFB<sub>2</sub>, taken with feeds, are metabolized in the liver in dairy animals and transformed into AFM<sub>1</sub> and

AFM<sub>2</sub> and pass into the milk from the mammary glands. The passing rate of AFB<sub>1</sub> to milk was reported as 0.18% in dairy cattle and 0.1% in sheep (Yentür and Er, 2011).

The problem of aflatoxin in dairy products occurs due to the presence of toxins in milk or milk powder and additives used in production or the development of toxigenic Aspergillus species in these products after milking (Kırdar, 2006). The type and composition of food, water activity, ambient temperature, relative humidity, gases in the environment, especially atmospheric oxygen and carbon dioxide level, storage time, storage conditions and harvest type have an effect on mold development and toxin formation (Ünlütürk, 1998; Bulca and Bircan, 2013). It has been reported that the distribution of toxin in milk is not homogeneous, AFM<sub>1</sub> changes to clot and whey in varying proportions, and the amount in the clot is higher due to its binding to casein (Bakırcı, 1995; Battacone et al., 2005).

Cube cheese is a local type of cheese which produced in many regions of our country. It is usually produced in closed family economy or in small enterprises by traditional methods. It is known as dish cheese, test cheese or pot cheese in some regions. The production, storage and marketing conditions of cube cheese are individual. The knowledge and skills of the manufacturer is effective on quality. Therefore, it is very difficult to provide a standard product composition (Kamber, 2005b; Üçüncü, 2013).

AFM<sub>1</sub> levels were determined in studies in different types of cheese in Turkey and other countries are given in Table 2 and Table 3. In the literature review there was no research on the presence of AFM<sub>1</sub> in Sivas cube cheeses.

The aim of this study is to investigate the presence of AFM<sub>1</sub> in the cube cheeses produced in Sivas as well as its regions and the evaluation of obtained data according to standards.

## Material and Method

### Material

The materials of this study were 90 cube cheese produced in Sivas and its region. Cheese samples were collected at regular intervals in November and December 2016 in various sales locations (home, market, public market, wholesale market) and the surrounding villages. Sterile stomacher bags were used for sampling. Samples were taken at least 200 g in aseptic conditions brought to the laboratory with cold chain application and analysed on the same day. Cheese samples were kept in the refrigerator during this process.

### Method

The level of AFM<sub>1</sub> was determined by ELISA (Enzyme-Linked Immunosorbent Assay) in cheese

products. HELICA Aflatoxin M<sub>1</sub> test kit (CAT. NO. 961AFLM01M-96) was used in the analyses.

### Preparation of cheese samples

It was weighed 1 g of a homogenized cheese sample into a centrifuge tube and added 5 ml of absolute methanol over 5 min. then mixed. The mixture was centrifuged at 5000 g for 5 min. After centrifugation, 0.5 ml of the supernatant was transferred to empty tubes and the methanol phase in the tube was evaporated under vacuum via vacuum oven (NÜVE EV018). The total of 0.5 ml of non-AFM<sub>1</sub>-free skimmed milk in the kit was added to the remaining viscous liquid and vortexed. After the process to collapse the contents of the tube allowed to stand for 5 min. The resultant extract was ready for use.

### Test procedure

From the standard solutions in the kit (0.5, 10, 25, 50, 100 ng/kg) and from each of the cheese samples, 200 µl was transferred to the microplate wells. The microplate was covered with ELISA film layer and left for 2 hours at room temperature and darkness. After this time, the wells were washed 3 times with the wash solution. After washing, 100 µl of enzyme conjugate was added to each well, at room temperature and in the dark for 15 min. and allowed to stand. Then the wells were washed 3 times with the wash solution. After washing, 100 µl of substrate is added to each well and 20 min. and allowed to stand under the same conditions. At the end of this period 100 µl stop solution was added to each well. The results were read at 450 nm via ELISA reader. Calibration curve was generated in the calculation of the absorbance values obtained and AFM<sub>1</sub> levels were calculated as ng/kg versus the absorbance of the samples.

### Statistical Analysis

The descriptive statistics of the AFM<sub>1</sub> values detected in the cube cheese samples and the relationships between the values were analysed in the SPSS 22.00 package program (SPSS, 2014).

## Results

Aflatoxin M<sub>1</sub> levels and percentage distribution of cube cheese samples produced in Sivas and region are given in Table 1.

According to the results of the analysis; AFM<sub>1</sub> were found in all 90 samples. The value of AFM<sub>1</sub> was determined as 2.16-53.94 ng/kg and the mean was determined as 6.36 ± 0.87 ng/kg. The level of AFM<sub>1</sub>, were found to be less than 5 ng/kg in 67.8% (61 samples) of the samples. It was found to be as 53.94 ng/kg in one sample (1.1%) (Table1).

Table1. AFM<sub>1</sub> values and percentage distribution in cube cheese

AFM <sub>1</sub> (ng/kg)	n	%	Minimum	Maximum	Mean±SE
1-5	61	67.8%	2.16	4.98	3.65±0.10
5-25	26	28.9%	5.10	20.89	7.98±0.71
25-50	2	2.2%	43.25	45.64	44.44±1.19
50-100	1	1.1%	53.94	53.94	53.94
100>	-	-	-	-	-
Total	90	100.0%	2.16	53.94	6.36±0.87

SE: Standard error

Table 2. The level of AFM<sub>1</sub> in cheeses from different countries researches (ng/kg)

Country	n	n <sub>1</sub> (%)	n <sub>2</sub> (%)	AFM <sub>1</sub> levels (min-max)	References
Southern Spain	35	16(44.7)		20-200	Barrios et al. (1996)
Brazil	75	56(74.7)	20(26.7)	20-692	Prado et al. (2000)
Libya	20	15(75)		110-520	Elgerbi et al. (2004)
Italy	265	44(16.6)		50-250	Montagna et al. (2008)
Brazil	88	40(46.4)	2		Prado et al. (2008)
Iranian	210	93(80.1)	(24.2)	52.1-785.4	Fallah et al. (2009)
Kuwait	40	32(80)	13		Dashti et al. (2009)
Egypt	150	50	-	51.6-182	Amer and İbrahim (2010)
Iranian	50	30(60)	3(6)	40.9-374	Tavakoli et al. (2012)
Lebanon	111	(67.56)			Elkak et al. (2012)
Brazil	90	18(60)	8(26.7)		Trombete et al. (2014)
Iranian	80	69(86.3)	11(13.8)	14.3-572.1	Rahimi (2014)
Costa Rica	70	49	13		Chavarria et al. (2015)
Iranian	10	6(60)		5.8-21.2	Sohrabi and Gharahkoli (2016)
Iranian	40	(65,5)	4		Bahrami et al. (2016)
Baghdad	40	15(53.85)	10		Al Mossawei et al. (2016)
Egypt	30	13	2	12.5-74.23	Tahoun et al. (2017)
Iranian	100	52(52)	8(8)	50.2-424.4	Sharifzadeh et al. (2017)

n: Sample number; n<sub>1</sub>: Positive sample; n<sub>2</sub>: Example exceeding the limit; \*Limit value: 50 ng/L; EC (2010)Table 3. AFM<sub>1</sub> levels determined in different types of cheese researches in Turkey (ng / kg)

Province	n	n <sub>1</sub> (%)	n <sub>2</sub> (%)	n <sub>3</sub> (%)	AFM <sub>1</sub> levels (min-max)	References
Van	50	n.d.				Kıvanç (1990)
İstanbul	75	36(45.2)		1	60-510	Dağoğlu et al. (1995)
Konya	240	n.d.				Gürbüz et al. (1999)
Ankara	150	n.d.				Kardeş (2000)
Bursa	57	(89.7)	7(12.28)	1	40-810	Oruç and Sonal (2001)
Marmara	110	101(91.8)	6		10-2000	Seyrek (2001)
İstanbul	186	121(65)	35(19)		40-4890	Ayçiçek et al. (2002)
İstanbul	15	15(100)	(13,3)		16-713	Özmenteşe (2002)
Bursa	125	86(68.8)	19(22.09)	9(10.46)	10-740	Günşen and Büyükyörük (2003)
Ankara	25	14(56)	1		10-400	Çetin (2004)
Ankara	400	327(81.7)	110(27.5)			Sarımehmetoğlu et al. (2004)
Erzurum	63	28(44.4)			7-202	Gürses et al. (2004)
Erzurum	50	47(94)	3(6)			Başkaya (2004)
Ankara	196	177	19			Ayçiçek et al. (2005)
Diff. province	600	30(5)	6(1)		100-800	Yaroğlu et al. (2005)
Van	110	83	68		100-7260	Tekinşen and Tekinşen (2005)
Kars	60	10			51-115	Kamber (2005a)
Konya	150	123	47		52-860	Özturan (2005)
Amasya	50	50(100)	1(2)			Alkan and Gönülalan (2006)
Aydın	25	25(100)	1(4)		40-250	Kök (2006)
Ankara	38	11(28.21)			78.2-188.4	Gürbay et al. (2006)
Sarıkamış	60	48	12			Kireççi et al. (2007)
Diff. province	132	109(82.6)	36(27.3)		50-690	Tekinşen and Eken (2008)
Diff. province	105	17	7(28)		51-400	Yapar et al. (2008)
Şanlıurfa	64	4(6,25)			51.1-99.6	Ardıç et al. (2008)
Erzurum	193	159(82.4)	60		52-860	Ardıç et al. (2009)
Diyarbakır	90	42(46.67)	13(14.4)			Erkan et al. (2009)
İstanbul	80	41(51.3)			52-2520	Hampikyan et al. (2010)
Maraş	46	32(69.6)			60-1200	Turgay et al. (2010)
Erzurum	304	216(71.1)	63(20.7)	30(9.9)	51-860	Atasever et al. (2010)
Erzincan	64	31	11			Gücükoğlu et al. (2010)
Samsun	50	25(50)			19.6-41.9	Aksoy et al. (2010)
Şanlıurfa	50	14(28)	5(10)		20-2000	Filazi et al. (2010)
Kayseri	60	38(63)	3		12-378	Ertaş et al. (2011)
Ege region	200	66	8		0.24-837.5	Eroğlu (2011)
İzmit	185	123(66.5)	32	10	12.3-760.4	Dinçoğlu et al. (2012)
Burdur	45	40(88.9)	7(15.6)	3(6.7)	55-600	Kocasari et al. (2012)
Diff. province	100	10				Dinçel et al. (2012)
Karadeniz reg	147	144(97.96)		16(11.1)	15-3774	Gül and Dervişoğlu (2014)
Kıbrıs	128	(21.7)			0.00-16.6	Öztürk et al. (2014)
Şanlıurfa	50				103.2	Temamoğulları and Kanıcı (2014)
Diff. province	166	70		5	50-2100	Bakırdere et al. (2014)
Ankara	27	25(92.6)			7.3-84.4	Sarıca et al. (2015)
Diff. province	60	25(41.7)			16-136	Kolucaçık et al. (2015)
Diff. province	100	52	19		10.6-702	Özgören and Seçkin (2016)

n: Sample number; n<sub>1</sub>: Positive sample; n<sub>2</sub>: >250 ng/kg; n<sub>3</sub>: >500 ng/kg; n.d.: Not detected

## Discussion and Conclusion

In this study, the presence and residual level of AFM<sub>1</sub> was investigated in locally produced cube cheeses in Sivas and the region. For this purpose, 90 cube cheese samples collected from the city center and surrounding villages were used as material. Aflatoxin analysis was performed by ELISA method.

According to the analysis findings, AFM<sub>1</sub> was detected in all of the cube cheese samples. AFM<sub>1</sub> values were determined as minimum 2.16, maximum 53.94 and average 6.36±0.87 ng/kg. AFM<sub>1</sub> level was lower than 5 ng/kg in 61 samples (67.8%). The levels of AFM<sub>1</sub> were determined between 5.10-20.89 ng/kg in 26 samples (28.9%), and between 43.25-45.64 ng/kg in 2 samples (2.2%). Toxin level was determined as 53.94 ng/kg in 1 sample (1.1%) (Table1).

In studies conducted in various countries, the levels of AFM<sub>1</sub> were determined in different types of cheeses between 0.6-6920 ng/kg. In this study, the level of AFM<sub>1</sub> determined in cube cheeses was similar to the findings of some researchers (Sohrabi and Gharahkoli, 2016; Tahoun et al., 2017), but was found lower than many other research results (Table 2).

In studies conducted in different years in our country, AFM<sub>1</sub> level in various types of cheeses was determined between 0.24-8375 ng/kg. The level of AFM<sub>1</sub> which determined in cube cheeses was found lower than the results of these researchers. The study data do not match the findings of the research (Kıvanç, 1990; Gürbüz et al., 1999; Kardeş, 2000), which reported that AFM<sub>1</sub> residue was not found in the cheese samples they examined (Table 3).

There are factors affecting aflatoxin level in cheese which are the hygienic quality of cheese milk, level of contamination, cheese type, number of samples, production technique, degree of fermentation, clot cutting size and analysis methods (Battacone et al., 2005; Bulca and Bircan, 2013; Üçüncü, 2013). The difference in between study results may be due to these factors. In addition, the fact that the studies were carried out in different years and in different regions probably contributed to the different results.

When the research findings are examined only one sample of the analyzed cube cheeses did not comply with the EU reported limit value (50 ng/kg) in terms of AFM<sub>1</sub> residue (EC,2010). The evaluation could not be made since there is no limit value regarding the AFM<sub>1</sub> level in cheese in the Turkish Food Codex (TGK, 2011).

As a result of it will be benefit in terms of food safety and public health since the detection of AFM<sub>1</sub> in all of the samples will be a potential risk for the consumer, by preventing mold contamination in ready-made food and feedstuffs, applying the standard method in making local cheeses, re-determining the limit value of AFM<sub>1</sub> for cheese with the regulation to be made in the Turkish Food Codex notification and regular inspections.

## Acknowledgements

This work is supported by the Scientific Research Project Fund of Sivas Cumhuriyet University under the project number 'V-024'.

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