



Seasonal Investigation of Aflatoxin M1 Level in Afyon Tulum Cheese

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ARTICLE INFO	ABSTRACT
<p><i>Article type:</i> Research Paper</p>	<p>Introduction: The milk produced from dairy animals fed with feed intake aflatoxin may include Aflatoxin M1 (AFM1). Dairy products, produced with milk containing AFM1 represent an important problem in terms of public health.</p>
<p><i>Article History:</i> Received: 25 Jul 2022 Accepted: 25 Aug 2022 Published: 01 Sep 2022</p>	<p>Methods: This study investigated AFM1 in Afyon Tulum cheese (ATC) taken in Afyonkarahisar province. For analysis, 80 samples of Afyon Tulum cheese were collected and stored at -20°C. All samples were then analyzed using a commercial Aflatoxin M1 ELISA test kit.</p>
<p><i>Keywords:</i> Dairy products Afyon tulum cheese Aflatoxin M1</p>	<p>Results: AFM1 was found between 0.007-0,017 ug/kg in spring-summer season and between 0.006-0,041 ug/kg in autumn-winter season. AFM1 levels were found under the Turkish Food Codex (TFC) limit (0,05 µg/kg) in all samples.</p>
	<p>Conclusion: It is recommended that milk containing AFM1 should not be used in production and the feeds used in animal feeding and storage conditions should be checked.</p>

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Introduction

Milk is the first nutrient consumed by humans and other mammals after birth. Rich in content and complex structure, milk is one of the essential nutrients for human nutrition. Milk is converted into durable products such as cheese or yoghurt to extend storage time. Cheese is the most popular of these products (1).

White, cheddar and Tulum cheese are among the most produced cheeses in Turkey. In addition to these cheeses, there are also many local kinds of cheese. Tulum cheese is the most known and made among the local cheeses. According to the Turkish Food Codex (TFC), Tulum cheese is a product produced by fermenting the curd obtained by coagulating with rennet, crumbling and salting it. It is a cheese that is then pressed into a suitable packaging material or leather overalls and consumed after ripening (2). Tulum cheese is produced in many Turkey regions, except the Thrace region. Tulum cheese is named differently according to areas and production methods. For example; Dıvle, Erzincan, Erzurum, Çimi and İzmir tulum cheeses (3, 3, 4). Another traditionally made cheese with its unique

production technology is Afyon Tulum cheese (5).

The presence of Aflatoxin M1 (AFM1) in milk and milk products poses a problem worldwide, especially in developing countries (6). Aflatoxins are toxic fungal metabolites generally produced by *Aspergillus parasiticus*, *A. flavus* and *A. nomenus* and occur in foods and animal feeds. When ruminants consume feed contaminated with Aflatoxin B1 (AFB1) during the lactation period, this toxin is metabolized in the digestive system and causes the formation of AFM1 in milk (7). Aflatoxins have severe adverse effects on human and animal health. It causes liver damage, immune system suppression, tumour formation, and teratogenic, mutagenic and carcinogenic effects (8).

Material & Method

Afyon Tulum Cheese Samples

This study collected Afyon Tulum cheese samples between December 2019 and November 2020 in Afyonkarahisar province. Eighty Afyon Tulum cheese samples (40 samples autumn-winter season and 40 samples spring-summer season) were collected, belonging to different sales

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points. The samples were stored at -20°C until analysis.

ELISA Kit

A commercial ELISA test kit (Bio-Shield M1 ES, Larissa, Greece) was used in the study. Aflatoxin M1 detection limit is $0.005 \mu\text{g}/\text{kg}$.

Preparation and Extraction Process

Dichloromethane (DCM) was used for extraction. For this purpose, 8 ml of DCM was added to a 2 g cheese sample and mixed. It was then incubated at room temperature ($21 \pm 2^{\circ}\text{C}$) for 30 minutes. After incubation, the tubes were centrifuged at $3000 \times g$ for 10 minutes at $21 \pm 2^{\circ}\text{C}$. The extractant was transferred to a different tube with 4 ml and evaporated at 60°C under N_2 . Then 0.5 ml of methanol (100%), 0.5 ml of distilled water and 2 ml of hexane were added to each sample. The tubes were mixed and centrifuged at $3000 \times g$ for 10 minutes. After centrifugation, top of the hexane layer and the bottom of the methanolic-aqueous phase were removed with the help of a pasteur pipette. The extract was diluted 1/10 with AFM1-free milk from the kit. $100 \mu\text{L}$ of each sample was used for ELISA measurement.

Preparation of ELISA Plates

All reagents of the kits to be used were brought to room temperature before use. $100 \mu\text{L}$ of standards and extracted samples were added to the wells. The wells were covered with a transparent film, shaken manually for 30 seconds, and incubated for 45 minutes at room temperature. The wells were washed four times with 1X wash buffer. After washing, $100 \mu\text{L}$ of fixation solution was added to all wells. The wells were covered with a transparent film, shaken by

hand for 30 seconds and incubated for 15 minutes at room temperature. The wells were washed four times with 1X wash buffer. $100 \mu\text{L}$ of TMB Substrate was added to all wells. Finally, the wells were covered with a transparent film, shaken manually for 30 seconds, and incubated for 15 minutes at room temperature for colour development. After incubation, $100 \mu\text{L}$ of Stop solution was added, and absorbance was read.

ELISA Reading and Evaluation

Absorbance reading was done at 450 nm in the prepared plate ELISA Reader (ThermoFisher, Multiskan-go, Vantaa, Finland), and the results were evaluated with the program belonging to the kit (Prognosis Data Reader).

Results & Discussion

AFM1 standard results (0, 0.005; 0.01; 0.025; 0.05, 0.1 and $0.25 \mu\text{g}/\text{kg}$) are shown in Figure 1. In the study, a total of 80 Afyon Tulum cheese samples (40 autumn-winter seasons, 40 spring-summer seasons) belonging to different sales points were collected. As a result, AFM1 was detected in 87.5% of the samples of the Autumn-Winter period; 97.5% in the spring-summer period samples. However, AFM1 was not detected above the TFC limit of $0.05 \mu\text{g}/\text{kg}$ in the Tulum cheese samples (Table 1). In addition, while the average of the samples belonging to the autumn-winter period was $0.02 \mu\text{g}/\text{kg}$, the average of the samples belonging to the spring-summer period was determined as $0.01 \mu\text{g}/\text{kg}$. According to the results, the AFM1 level in the autumn-winter period was higher than in the spring-summer period.

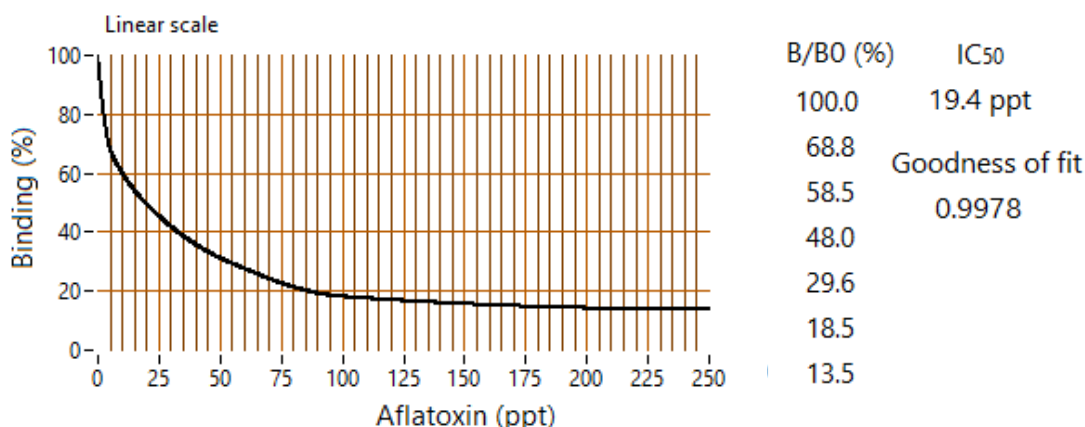


Figure 1. Standards of Aflatoxin M1

In similar studies, Sarımeahmetoğlu et al. (2004) detected AFM1 in 81% of Tulum cheese and reported that 24% of the samples were above the Turkish Food Codex AFM1 limit (9). Hampikyan et al. (2010) AFM1 was detected in 55% of Tulum cheese samples (between 0.057-1.36 ng/kg levels) (10). They also reported that 10% of the Tulum cheese samples were above the TFC limit. In the study of Gücükoğlu et al. (2010) did not detect AFM1 in Tulum cheese (11). Ertas et al. (2011) AFM1 was detected in 16 of 20 Tulum

cheese samples (in the range of 13.0-378.0 ng/kg). In two samples, AFM1 detections were above the TFC limit (12). İşleyici et al. (2011) AFM1 determined in amounts varying between 5.15 ng/kg and 26.44 ng/kg (13). Ayyıldız (2012) AFM1 detected 113.07 ng/kg in one (14.3%) Tulum cheese sample (14). Bakırdere et al. (2014) found AFM1 detected in 18.75% (3) of 16 Tulum cheeses (0.05-0.10 µg/kg) (15). They did not find the AFM1 level of the samples above the limit set by the TFC (0.05 µg/kg) (2).

Table 1 Aflatoxin M1 Levels in Afyon Tulum Cheese

	Spring-Summer 40)	Season (n: 40)	Autumn-Winter (n:40)	Season	Total (N:80)	
	n	%	n	%	n	%
< 0.005	1	2.50	5	12.50	6	7.25
≥0.005 - <0.01	12	30.00	3	7.50	15	18.75
≥0.01 - <0.02	27	67.50	28	70.00	55	68.75
≥0.02 - <0.03	0	0.00	3	7.50	3	3.75
≥0.03 - <0.05	0	0.00	1	2.50	1	1.25
≥0.05	0	0.00	0	0.00	0.00	0.00
Min*	0.007		0.006			
Max	0.017		0.041			
Average*	0.01		0.02		0.014	

* Samples above the detection limit (0.005 µg/kg) were calculated.

In all studies, AFM1 in different rates and levels were detected in Tulum cheeses. Although AFM1 was detected in Tulum cheeses in this study, the AFM1 level was found below the TFC maximum limit. Similarly Gücükoğlu et al. (2010), İşleyici et al. (2011), and Bakırdere et al. (2014) found the AFM1 level in Tulum cheese to be below the maximum limit of the TFC (11, 13, 15). On the other hand, Sarımeahmetoğlu et al. (2004), Ertas et al. (2011), Hampikyan et al. (2010), and Ayyıldız (2012) reported that they determined the level of AFM1 in Tulum cheeses above the TFC limit (9, 10, 12).

Differences between studies can be attributed to the Aflatoxin content of milk and animal feed used in production. Inappropriate stages may cause aflatoxin contamination in feed, harvesting, and storing feeds. In addition, seasonal air temperature changes also affect the formation and amount of Aflatoxin in feed. In the study, the AFM1 level in the autumn-winter period's Tulum cheese samples was higher than in the spring-summer period. This situation may be related to the consumption of animals in closed feeding and stored feed materials.

Conclusion

Aflatoxin-free milk should be used in the production of Tulum cheese. Routine checks for

Aflatoxin should be made for milk and cheese. It is recommended to prevent mould formation in the processing, transportation and storage stages of the feed used in animal feeding, starting from the growing and harvesting stage. The necessary hygienic conditions must be observed in the production area for the hygienic quality of milk and dairy products. The Farm to Fork principle should control the products at all stages. Manufacturers and consumers should be informed about Aflatoxin.

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Conflicts of Interest

The authors declares no potential conflict of interest.

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