

## Determination of Aflatoxin Levels in Raw Milk, Cheese and Dehulled Hazelnut Samples Consumed in Samsun Province, Turkey <sup>[1]</sup>

Abdurrahman AKSOY \*  Oguzhan YAVUZ \* Dilek GUVENC \*  
Yavuz Kursad DAS \* Goknur TERZİ \*\* Suleyman CELİK \*\*\*

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\* Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, TR-55139 Samsun - TURKEY

\*\* Department of Food Hygiene and Technology, Faculty of Veterinary Medicine, Ondokuz Mayıs University, TR-55139 Samsun - TURKEY

\*\*\* Department of Pharmacology, Faculty of Medicine, Ondokuz Mayıs University, TR-55139 Samsun - TURKEY

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### Summary

Thirty six samples of raw cows' milk, 50 samples of milk products (25 samples each of fresh white cheese and Kashar cheese), and 50 samples of dehulled hazelnut were randomly collected from the Samsun province of Turkey. The dairy products and hazelnut samples were analyzed for the presence of aflatoxin M1 (AFM1) and aflatoxin B1 (AFB1), respectively, by microtiter-plate enzyme-linked immunosorbent assay (ELISA). The incidence of AFM1 contamination in the samples of raw cow milk, fresh white cheese and Kashar cheese were 61%, 12%, and 80%, respectively. AFB1 contamination was detected in 43 (86%) of the dehulled hazelnut samples (ranging from less than 1 to 11.3 µg kg<sup>-1</sup>). The AFM1 levels determined in the samples of milk and dairy products were lower than the limit set by the Turkish Food Codex and European Union, whereas the AFB1 levels of two dehulled hazelnut samples exceeded the legal limit.

**Keywords:** Aflatoxin, Milk, Dairy products, Dehulled hazelnut, ELISA, Samsun

## Samsun Çevresinde Tüketime Sunulan Çiğ Süt, Peynir ve Fındık Örneklerinde Aflatoksin Seviyelerinin Belirlenmesi

### Özet

Otuz altı adet çiğ inek sütü, 50 adet süt ürünü (25'er adet taze beyaz peynir ve kaşar peyniri) ve 50 adet fındık örneği Samsun çevresinden rastgele toplandı. Süt ürünü ve fındık örnekleri sırasıyla aflatoksin M1 (AFM1) ve aflatoksin B1 (AFB1) yönünden ELISA testi kullanılarak analiz edildi. Çiğ inek sütü, taze beyaz peynir ve kaşar peyniri örneklerinde AFM1 ile bulaşma oranları sırasıyla %61, %12 ve %80 olarak belirlendi. Fındık örneklerinin 43 adedinde (%86) AFB1 tespit edildi (<1 ile 11.3 µg/kg<sup>-1</sup> arasında). Süt ve süt ürünlerinde tespit edilen AFM1 miktarlarının Türk Gıda Kodeksi ve Avrupa Birliği tarafından belirlenen en yüksek tolere edilebilir limitlerin altında olduğu, bununla birlikte 2 fındık örneğinde AFB1 miktarının yasal limitlerden yüksek olduğu görüldü.


**Anahtar sözcükler:** Aflatoksin, Süt, Süt ürünleri, Fındık, ELISA, Samsun

### INTRODUCTION

Mycotoxicosis, which can occur in both industrialized and developing countries, arises when environmental, social and economic conditions combine with weather

conditions (humidity and temperature) that favor the growth of moulds <sup>1</sup>. The four main aflatoxins are B1 (AFB1), B2 (AFB2), G1 (AFG1) and G2 (AFG2), and were

 **İletişim (Correspondence)**

 +90 362 3121919/ 2816

 aaksoy@omu.edu.tr

named on the basis of their fluorescence (blue or green) under UV light and their relative chromatographic mobility during thin-layer chromatography <sup>2</sup>. The foremost food sources of aflatoxins include grains (particularly corn, sorghum and millet), peanuts, beans and tree nuts, including almonds and pistachios hazelnuts <sup>3</sup>.

Exposure of animals to AFB1 occurs mainly by the ingestion of contaminated feeds. In the liver, ingested AFB1 is biotransformed by the hepatic microsomal cytochrome P450 into aflatoxin M1 (AFM1), which is then excreted into the milk of lactating animals <sup>4,5</sup>. In dairy cows the amount of AFM1 excreted into milk can be up to 3% of the AFB1 intake and is affected by milk yield <sup>6</sup>. The consumption of milk and milk products by human populations is quite high, particularly by infants and young children, thereby increasing the risk of exposure to AFM1 <sup>7</sup>. The European Commission (EC) and Turkish authorities have adopted 50 ng kg<sup>-1</sup> as the maximum residue limit (MRL) for raw milk, heat-treated milk and milk used for the manufacture of milk-based products. However, the MRL is 25 ng kg<sup>-1</sup> for infant milk <sup>8,9</sup>.

Globally, the leading hazelnut producers are Turkey (73% of market share), followed by Italy (14%), the United States (4%), Spain (3%) and others (6%) <sup>10</sup>. In Turkey, hazelnuts are traditionally sun-dried and thus may be subject to mould growth and subsequent aflatoxin formation due to prolonged drying under humid or rainy conditions, similar to the process in other nuts <sup>11</sup>. The EC and Turkish governments have set the MRL for total aflatoxins in hazelnut, which is offered for direct human consumption, at 10 ng kg<sup>-1</sup> <sup>8,9</sup>.

The aim of this study was to determine the occurrence and levels of aflatoxins in dairy products and dehulled hazelnut samples consumed in Turkey.

## MATERIAL and METHODS

Thirty six samples of raw cow's milk, 50 samples of milk products (25 samples each of fresh white cheese and Kashar cheese) and 50 dehulled hazelnut samples were randomly collected from the Samsun province in Turkey. The samples of dairy products and hazelnut were analyzed for the presence of aflatoxin AFM1 and AFB1, respectively, by ELISA.

The analysis was performed according to the procedures described by R-Biopharm GmbH <sup>12</sup>. Milk samples were centrifuged for 10 min at 3.500 rpm and 10°C for degreasing. After centrifuging, the upper layer of cream was removed completely by aspiration using a

Pasteur pipette. Skimmed milk (defatted supernatant) was used directly in the test (100 µl per well). The limit of detection (LOD) for AFM1 in milk was <10 ng l<sup>-1</sup>.

Samples of two grams of white or Kashar cheese and 40 ml of dichloromethane were used for extraction of AFM1. The suspension was filtered and a 10 ml aliquot was evaporated under a nitrogen stream. The extraction procedure was repeated with 0.5 ml of phosphate buffered saline (PBS), 0.5 ml of methanol and 1 ml of heptane. The extract obtained was subsequently centrifuged for 15 min at 2500 rpm and 15°C. The methanol layers were used for testing. The LOD for AFM1 in white and Kashar cheese was <100 ng kg<sup>-1</sup>.

Two grams of powdered hazelnut samples were shaken for 10 min with 7 ml of methanol for AFB1 extraction. Then two milliliters of filtrate were transferred into a screw-top centrifuge vial, 2 ml of distilled water and 3 ml of dichloromethane were added and mixed for 5 min, and the solution was centrifuged for 5 min at 3250 rpm and 15°C. The upper aqueous layer was removed and the entire dichloromethane layer was used for the subsequent steps. Firstly, the dichloromethane layer was evaporated at 50-60°C. The extraction procedure was repeated with 0.4 ml PBS and 1.5 ml heptane, as described above. The upper heptane layer was removed, and the methanol layer was used for AFB1 testing. The LOD for AFB1 in hazelnut samples was 625 ng kg<sup>-1</sup>.

ELISA was carried out on a Digital and Analog Systems microplate reader (Rome, Italy). RIDASCREEN Aflatoxin ELISA kits (R-Biopharm AG, Darmstadt, Germany) were used to determine the levels of AFM1 and AFB1. The basis of the test was the detection of specific antigens using the antigen-antibody reaction in the presence of an enzyme. The wells in the microtiter strips were coated with antibodies specific to AFM1 or AFB1. Measurements were carried out photometrically at 450 nm, and the absorption was inversely proportional to the concentration of aflatoxins present in the samples.

## RESULTS

AFM1 contamination in the dairy products (raw fresh milk, white fresh cheese and Kashar cheese) is shown in *Table 1*. The AFM1 levels were lower than the maximum levels (cheese: 500 ng kg<sup>-1</sup>; milk: 50 ng kg<sup>-1</sup>) allowed by the Turkish Food Codex <sup>9</sup>. The AFB1 levels of dehulled hazelnut samples are shown in *Table 2*. They exceeded the legal limits of the EC (5 µg kg<sup>-1</sup>) <sup>8</sup> in only two samples.

**Table 1.** Aflatoxin M1 levels in samples of milk, white cheese, and Kashar cheese**Tablo 1.** Süt, beyaz peynir ve kaşar peyniri örneklerinde aflatoksin M1 seviyeleri

AFM1 (ng kg <sup>-1</sup> )	Milk		White Cheese		Kashar Cheese	
	n	%	n	%	n	%
Not detected	14	38.89	22	88.00	5	20.00
Detected	22	61.11	3	12.00	20	80.00
<1	7	19.44	-	-	-	-
1-10	15	41.67	1	4.00	3	12.00
10-50	-	-	2	8.00	13	52.00
50-100	-	-	-	-	3	12.00
100-250	-	-	-	-	1	4.00
>250	-	-	-	-	-	-
Total (n)	36	100.00	25	100.00	25	100.00
X±Sx	2.32±0.40		19.67±6.53		41.93±10.69	
Legal limit ng kg <sup>-1</sup>	50 *		500 **		500 **	

\* According to Turkish Food Codex and Commission Regulation of EC [8,9]  
\*\* According to Turkish Food Codex [9]

**Table 2.** Aflatoxin B1 levels of dehulled hazelnut samples**Tablo 2.** Fındık örneklerinde aflatoksin B1 seviyeleri

AFB1 (µg kg <sup>-1</sup> )	n	%
Not detected	7	14.00
Detected	43	86.00
<1	26	52.00
1-5	15	30.00
5-10	1	2.00
>10	1	2.00
Total (n)	50	100.00
X±Sx	1.72±0.32	
Legal limit µg kg <sup>-1</sup> *	5	

\* According to Commission Regulation of EC [8]

## DISCUSSION

Monitoring programs for aflatoxin risk assessment in foodstuffs have been implemented in many countries, including Turkey, Italy, India and Spain <sup>1,7,13-19</sup>.

Aycicek et al.<sup>1</sup> analyzed 223 samples of dairy products, 51 dehulled hazelnut samples and 40 cacao hazelnut creams in Turkey. Approximately 91.5% of white cheese samples were contaminated with AFM1, which is considerably higher than the result for our study (12%). However, 88.7% of the Kashar cheese samples from that study were positive for AFM1, similar to the result (80%) of the present study. Moreover, the AFB1 level of the dehulled hazelnut samples in that study (84.3%) was in parallel with our result (80%).

Tekinsen and Eken <sup>13</sup> investigated 100 ultra high temperature (UHT) milk and 132 Kashar cheese samples in Turkey for the presence of AFM1. It was determined that 67% of the UHT milk samples and 82.6% of the

Kashar cheese samples were positive for AFM1. The results are similar to the results obtained from the samples of milk (61%) and Kashar cheese (80%) in the present study. However, in stark contrast, the AFM1 levels in 31 (31%) samples of UHT milk and 36 (27.3%) samples of Kashar cheese in the above study <sup>13</sup> exceeded the maximum tolerable limit of the EC and the Turkish Food Codex.

Gürses et al.<sup>14</sup> analyzed 77 samples of cheese in Turkey and found approximately 44% of samples to be positive for AFM1 residues. However, none of the levels was above the legal limit of the Turkish Food Codex <sup>9</sup>.

Gürses <sup>15</sup> investigated 28 hazelnut, 24 walnut, 18 peanut, 13 almond and 11 roasted chickpea samples for aflatoxin contamination in Turkey. Nine of the 28 hazelnut samples were contaminated. The highest level of aflatoxin in those samples was 113 µg kg<sup>-1</sup> and the mean concentration of aflatoxin was 33.4 µg kg<sup>-1</sup>.

Bognanno et al.<sup>16</sup> examined 240 milk samples from dairy ewes in Italy. Eighty one percent of the samples were positive, which is higher than the result obtained in the present study (61%). Three samples with contamination above the legal limit (50 ng l<sup>-1</sup>) were detected in that study.

Rastogi et al.<sup>7</sup> investigated AFM1 contamination in 87 samples of infant milk products and liquid milk in India. An 87.3% contamination rate was detected in the samples. It was also determined that the level of contamination by AFM1 (65-1012 ng l<sup>-1</sup>) in infant milk products were higher than in liquid milk samples (28-164 ng l<sup>-1</sup>). Almost 99% of the contaminated samples had contamination exceeding the EC limits (50 ng l<sup>-1</sup>).

Nachtmann et al.<sup>17</sup> analyzed 316 milk samples in Italy and detected two samples (0.6%) with levels greater than the limits set out in the regulations. Five samples (1.6%) had a contamination level of 50 µg l<sup>-1</sup>. Only two of the 316 samples were above the legal limit, whereas there were five samples at the threshold of the legal limit, which parallels the results of the present study.

Blesa et al.<sup>18</sup> examined 58 food samples for aflatoxin contamination in Spain. Three positive samples, which were hazelnut (0.42 and 0.52 µg kg<sup>-1</sup> for AFB1 and AFG1, respectively), nut cocktail (0.29 and 0.47 µg kg<sup>-1</sup> for AFB1 and AFG1, respectively), were detected. Except for two samples, the contamination levels detected in that study were below the limits of EU standards.

Bircan et al.<sup>19</sup> analyzed food samples for export from Turkey for total aflatoxin concentrations. Three hundred and thirteen of 2.643 dried fig samples, two of 80 hazelnut samples, 17 of 28 pistachio samples, five of 10 peanut samples, and 19 of 23 paprika samples were positive and within the ranges of 0.2-162.76, 5.46-6.55, 2.31-63.11, 0.75-26.36 and 1.79-6.55 µg kg<sup>-1</sup>, respectively.

In the present study, the AFM1 levels detected in samples of milk and cheese products were lower than the maximum tolerable limit set by the Turkish Food Codex and European Union standards, whereas the AFB1 levels of two dehulled hazelnut samples exceeded the legal limit. However, the incidence of AFM1 in milk and Kashar cheese was high. In addition, AFB1 was also detected in most of the dehulled hazelnut samples. Although the contamination level was low, these residues would probably be harmful for humans. Clearly, any risk assessment study of a naturally occurring toxic compound must be based on information about its occurrence, exposure and toxicology, as indicated in the literature<sup>1</sup>.

Therefore, it is concluded that the aflatoxin levels of dairy products and hazelnut samples should be monitored regularly and rigorously by the appropriate government agency and with mandatory public reporting. At the same time, it is imperative that producers, processors and consumers be educated about the hazards of these compounds and how to manage food products to minimise contamination. Consumer rights groups and consumers themselves should be vigilant and demand that producers, processors and government all work conscientiously to minimise the hazard of aflatoxin in animal and human foodstuffs.

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