

## Aflatoxin M1 levels in retail yoghurt and ayran in Erzurum in Turkey

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**Abstract:** This study was carried out to determine the presence and levels of aflatoxin M1 (AFM1) in yoghurt and ayran consumed in the province of Erzurum, Turkey. For this purpose, a total of 80 yoghurt samples and 80 ayran samples were randomly obtained from markets. The samples' AFM1 content and concentrations were examined by competitive ELISA method. AFM1 at detectable level (5 ng/kg) was found in 87.5% of the yoghurt samples and in 90.0% of the ayran samples. AFM1 levels in 16 (20%) yoghurt and in 11 (13.6%) ayran samples exceeded the maximum tolerable limit of the Turkish Food Codex. The positive incidence of AFM1 in the yoghurt and ayran samples ranged from 10 to 475 ng/kg and from 6 to 264 ng/kg, respectively. It was concluded that high AFM1 level is an important problem threatening public health in Turkey. Therefore, dairy products have to be controlled routinely for presence of AFM1 contamination by public health authorities.

**Key words:** Aflatoxin M1, yoghurt, ayran, ELISA

### Erzurum'da satışa sunulan yoğurt ve ayran örneklerindeki aflatoksin M1 düzeyleri

**Özet:** Bu çalışma, Erzurum İli'nde satışa sunulan yoğurt ve ayran örneklerindeki Aflatoksin M1 (AFM1) varlığını belirlemek için yapıldı. Bu amaçla, toplam 80 yoğurt örneği ve 80 ayran örneği marketlerden rastgele temin edildi. Örneklerin AFM1 içerik ve konsantrasyonları kompetitiv ELISA metoduyla araştırıldı. AFM1 düzeyinin yoğurt örneklerinin %87,5'i ve ayran örneklerinin ise %90,0'ında belirlenebilir limitlerin üzerinde olduğu (5 ng/kg) bulundu. AFM1 seviyesinin 16 (%20) yoğurt numunesinde ve 11 (%13,6) ayran numunesinde Türk Gıda Kodeksi limitlerini aştığı belirlendi. AFM1 düzeyi yoğurt örneklerinde 10 ile 475 ng/kg ve ayran örneklerinde 6 ile 264 ng/kg arasında değişti. Sonuç olarak yüksek AFM1 düzeyinin Türkiye'de halk sağlığını tehdit eden bir problem olduğu görüldü. Bundan dolayı süt ürünlerinin AFM1 kontaminasyonu yönünden sağlık otoriteleri tarafından rutin olarak kontrol edilmesi gerekmektedir.

**Anahtar sözcükler:** Aflatoksin M1, yoğurt, ayran, ELISA

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pergillus: *A. flavus*, *A. parasiticus* and to a lesser extent by *A. nomius*, which contaminates plants and plant products. When lactating mammals such as cows, sheep, and goats are fed with feedstuffs containing aflatoxin B1 (AFB1), this metabolite can be converted to aflatoxin M1 (AFM1) (1). The occurrence of AFM1 in milk, especially cow's milk, makes it a particular risk for humans because of its importance as a foodstuff for adults and especially for children. This toxin has been categorized by the International Agency for Research on Cancer (IARC) as a class 2B toxin, a possible human carcinogen (2). A tolerable daily intake of 0.2 ng/kg b.w. for AFM1 was calculated by Kuiper-Goodman (3). The intake of AFM1 from milk is calculated to be 6.8 ng/person per day for the European diet, and 0.7 ng/person per day for the Middle Eastern diet (4). Cultured dairy products are manufactured by heating milk and adding a starter culture to initiate the fermentation. Studies have not shown that there was a significant decrease in the AFM1 content of cultured dairy products, such as kefir and yoghurt (1).

To protect consumers several countries have established legislation to regulate the levels of AFB1 in feeds and AFM1 in milk and dairy products (5,6). In Turkish Food Codex (TFC) legal limits for AFM1 in both yoghurt and ayran are 50 ng/kg (7).

Yoghurt is a fermented dairy product gained by lactic acid fermentation of milk by the action of yoghurt starter bacteria and is the most popular product throughout the world (8). Ayran is a yoghurt drink produced in Turkey. It is traditionally manufactured by addition of water at a level of 30%-50% and salt at a maximum level of 1% to yoghurt (9).

Many researchers from different countries have carried out studies about the incidence of AFM1 in yoghurt. Kim et al. (10) showed that the incidence of AFM1 in yoghurt was 83%, with a mean concentration of 29 pg/g in Korea. Martins and Martins (11) in Portugal reported that the level of AFM1 in 18.8% of the samples (18 out of 96 samples) ranged between 11 and > 65 ng/kg. Galvano et al. (12) demonstrated that, in 114 samples of yoghurt, 91 (80.0%) contained AFM1 with levels ranging from 1 to 496.5 ng/kg. Later Galvano et al. (13) reported that in 120 yoghurt samples analyzed, 73 (61.0%) samples

were contaminated with AFM1 at lower levels (1-32.1 ng/kg). Sylos et al. (14) did not detect the presence of AFM1 in 30 yoghurt samples examined in Campinas, Brazil. According to these authors, the possible explanation for the absence of AFM1 is that cows in the Campinas area graze all year round. Another explanation could also be that the methodology used for the detection of AFM1 was the TLC method (14). Srivastava et al. (15) determined that, in 1 of the 5 yoghurt samples, the presence of AFM1 was detected at the 0.03 µg/kg level.

There is little information about the occurrence of AFM1 in yoghurt in Turkey. To our knowledge there is no study on the AFM1 content of ayran. The purpose of this survey was to determine the natural occurrence and levels of AFM1 in samples of yoghurt and ayran manufactured in Turkey and to compare the results with the maximum AFM1 tolerance limits that are accepted by the TFC.

A total of 80 samples of yoghurt and 80 samples of ayran were obtained randomly from markets between September 2007 and September 2008 in the province of Erzurum. The samples were transported to the laboratory in an insulated container at about 4 °C and analysed upon arrival.

AFM1 concentrations of the samples were analysed by competitive ELISA (RIDASCREEN Aflatoxin M1, R-Biopharm) as procedure described by R-Biopharm GmbH (16). The samples were evaluated according to the RIDAVIN computer program prepared by R-Biopharm, and the statistical analysis was performed by SPSS. According to the instructions for use of the RIDASCREEN kit, the lower detection limit was 5 ng/kg.

In this study, a total of 80 yoghurt samples and 80 ayran samples were analysed for AFM1 with the competitive ELISA. The occurrence and the distribution of AFM1 concentration in various ranges in yoghurt samples and ayran samples are presented in the Table.

AFM1 was determined over the detectable level in 87.5% (70/80) of yoghurt samples and in 90.0% (72/80) of ayran samples. The AFM1 levels were higher than the limits of the TFC (14) (50 ng/kg yoghurt and ayran) in 20.0% (16/80) yoghurt samples and in 13.6% (11/80) ayran samples. AFM1 amounts

Table. Occurrence and distribution of aflatoxin M1 in yoghurt and ayran samples.

Kind of samples	Samples tested (n)	Level of positive samples n (%)	Distribution of samples n (%)					> TFC legal limit >50 ng/kg	Positive samples (ng/kg)		
			<5*	5-25	26-50	51-100	>100		$\bar{x} \pm Sx$	Min.	Max.
			Yoghurt	80	70/80 (87.5)	10/80 (12.5)	21/80 (26.3)	32/80 (40.0)	6/80 (7.5)	10/80 (12.5)	16/80 (20.0)
Ayran	80	72/80 (90.0)	8/80 (10.0)	28/80 (35.0)	33/80 (41.3)	8/80 (10.0)	3/80 (3.8)	11/80 (13.6)	36.5 ± 34.84	6	264

TFC = Turkish Food Codex; ( ) = indicates percent;  $\bar{x} \pm Sx$  = Mean ± standard deviation; \* = distribution of negative samples.

of positive samples were determined in yoghurt samples and ayran samples as minimum 10 ng/kg, 6 ng/kg, maximum 475 ng/kg, 264 ng/kg, and mean  $66.1 \pm 99.5$  ng/kg,  $36.5 \pm 34.84$  ng/kg, respectively. The incidences of AFM1 in yoghurt and ayran samples were similar to the reported results by Kim et al. (10) and Galvano et al. (12). The contamination levels of AFM1 were clearly higher than those in the previous studies (10,13,15). The variations on the findings may be ascribable to the fact that milk to be processed may contain different levels of AFM1 according to the seasonal changes (17,18) and the different processing techniques and analysis methods (18,19). Moreover, differences in the hygiene and storage conditions at the dairies and retail points are other key factors affecting the variations of the results (20).

Some AFM1 levels obtained from yoghurt samples (16 samples; 20%) and ayran samples (11 samples; 13.6%) exceeded the legal limit of TFC (50 ng/kg), which may cause public health problems. Very high AFM1 levels (51 to > 100 ng/kg) in 20.0% of yoghurt samples and in 13.8% of ayran samples

are of great importance. The AFM1 contamination rates obtained from yoghurt samples were found to be higher than those obtained from ayran samples. The lower contamination rate of ayran than yoghurt might be due to the addition of about 30%-50% water to yoghurt in order to dilute for ayran production. In conclusion, the levels of AFM1 in yoghurt samples and ayran samples produced in Turkey were very high compared to those in other countries. Therefore, we can conclude that the incidence of AFM1 in yoghurt and ayran consumed in Turkey appears to be a serious public health hazard at the moment, since 20.0% of the yoghurt samples and 13.6% of ayran samples exceeded the legal limit (50 ng/kg) of AFM1 stated in TFC. So the public health authorities should take necessary measures, and the producers should be informed. Furthermore, the most effective way of controlling AFM1 in the food supply is to reduce contamination with AFB1 of raw materials and supplementary feedstuffs for dairy cattle. Preventive measures must be applied to reduce fungal growth and AFB1 formation in agricultural commodities intended for use as animal feeds.

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