

Aflatoxin M1 Contamination in Traditional Yoghurts Produced in Guilan Province, Iran

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Abstract

Aflatoxin M1 is a major carcinogenic compound that may be existed in dairy products. The aim of this study was to determine the occurrence of AFM1 in traditional yoghurt samples in Guilan Province (Northern Iran). Ninety samples of traditional yoghurts were collected during summer and autumn 2014. Enzyme linked Immunosorbent assay (ELISA) which is a rapid and sensitive method was used to determine the presence and levels of AFM1. 100% of the yoghurt samples were contaminated with 5 and 83 ng/kg of AFM1. In general, AFM1 in 20 samples (22.22%) were higher than the maximum tolerance limit (50 ng/kg) accepted by the European Union. It was therefore concluded that, high occurrence of AFM1 in yoghurt is a serious risk for public health.

Keywords: Aflatoxin M1, ELISA Traditional Yoghurt

1. Introduction

Aflatoxins are severe toxic secondary metabolite products of some *Aspergillus* spp. When animals consume feed contaminated with Aflatoxin B1, the toxin is metabolized by liver and is excreted as Aflatoxin M1 via milk. Aflatoxins are toxic compounds, mutagen immunosuppressive and carcinogen¹. People in developing countries are at risk of chronic exposure to aflatoxins through contaminated foods². Among aflatoxins, B1, B2, G1 and G2 are the major toxins. Aflatoxins, M1 and M2 are modified form of AFB1. According to Agency for Research on Cancer (IARC) B1, B2, G1 and G2 as Group 1 carcinogens, and M1 as Group 2. AFM1 display distribution variations according to season, geography and country.

The contamination level of AFM1 is different in hot and cold seasons³. The European Union proposes a maximum permissible level of 50 ng/kg AFM1 in dairy products⁴. When yoghurts are manufactured from AFM1 contaminated milk, the toxin could be detected, because AFM1 is a stable compound that persists against most of food processing stages⁵. Aflatoxins have sub-acute and chronic effects such as chronic hepatitis, liver cancer, jaundice, hepatomegaly in humans³. Traditional yoghurts are made in ranches or small dairy shops in Iran. There is little information about the occurrence of AFM1 in Traditional yoghurt in north of Iran⁵⁻⁷. Therefore, the aim of this study was to evaluate the presence of AFM1 in traditional yoghurts produced in Guilan Province (Northern Iran) by ELISA method.

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2. Materials and Methods

2.1 Samples

A total of 90 traditional yoghurt samples in each season from Guilan Province (Northern Iran) were collected randomly during summer and autumn 2014. The samples were transported to the laboratory and were pasteurized (80°C for 3 min). Then, the samples were cooled down to room temperature and diluted in 1:5 in PBS-buffer with pH: 7.2 and 100 µl of this solution was used for ELISA.

2.2 Determination of AFM1

AFM1 detection was performed using competitive ELISA using R-Biopharm AFM1 kit. The AFM1 standards and test samples (100 µl per well) in duplicate were added to the wells of a micro-titer plate precoated with antibodies for AFM1 and incubated at room temperature in dark for 60 min. Then added, 100µl of enzyme conjugate was added to the wells and plate was incubated again for 60 min at room temperature in dark. Then 50 µl enzyme substrate (urea peroxide) and chromogen solution (tetramethyl-benzidine) was added to the wells and incubated for 30 min in dark. At the end, 100 µl of stop solution were added to each well. The optical absorbance of each well was read at 450 nm with ELISA reader³. Statistical analysis of results was performed with SPSS 22 software.

3. Results and Discussion

The distribution of AFM1 was shown in Table 1. Of the ninety samples analyzed, 100% samples were found to be contaminated with AFM1. Twenty samples (22.22%) failed to reach the desired level of the European Union (50ng/kg). The aflatoxin M₁ contamination levels were 5-83ng/kg with the mean value of 32.11ng/kg.

The range of contamination levels varied in two seasons (Table 2). AFM1 in summer ranged from 5-80.5ng/kg and in autumn ranged from 5-83ng/kg with the mean values 30.7 and 33.53ng/kg respectively. The highest

Table 1. Occurrence of AFM1 in traditional yoghurt samples

AFM ₁ levelng/kg	No. of samples	(%)	Range
< 25	41	45.55	5-23.5
26-49	29	32.22	26.5-48
≥ 50	20	22.22	50-83
Total Samples	90	100	5-83

Table 2. Contamination levels in summer and autumn

Range	Mean	Positive samples	Season
5-80.5	30.7	100%	45 Summer
5-83	33.53	100%	45 Autumn

mean concentration of aflatoxin M1 registered in autumn 83ng/kg, while in summer samples highest concentration of aflatoxin M1 was 80.5ng/kg.

AFM1 contamination in dairy products is a worldwide problem threatening public health in all areas of the world. The contamination of dairy products with AFM1 display variations according to season, geography and country³. Higher level of AFM1 in traditional yoghurt might be due to high level of AFB1 in foodstuff⁵. Various surveys were performed in order to determine the AFM₁ levels in yoghurt. Authors in⁷ reported that all tested traditional yoghurt samples in Guilan province were contaminated with AFM1 (4.2-78.9ng/kg) and 13.33% of the tested samples had contamination levels above the European regulation (50ng/kg). According to⁸ in Portugal, stated that ninety six local yoghurt samples tested by HPLC was found, ranging 19-98ng/kg. Authors in⁵ reported 96.25% of the yoghurt samples (40 industrial, 40 traditional) were contaminated with AFM1 in concentration levels ranging from <5 to 91ng/kg. According to⁹, in Ankara detected AFM1 contamination in 62.88% yoghurt samples, ranging from 50-800ng/kg. In another study¹⁰, in Turkey, 177 yoghurt samples (104 ordinary yoghurt, 21 fruit yoghurt and 52 of strained (Torba) yoghurt) were tested for AFM1 by ELISA method. 11.53% of ordinary yoghurt, 9.52% of fruit yoghurt, and 21.15% of strained yoghurt had higher AFM1 (50ng/kg). Authors in⁶, reported, 100% pasteurized yoghurt samples and local yoghurt collection from Mazandaran Province (Northern Iran) were positive with concentrations of AFM1 2.1-61.7 and 7-53ng/kg respectively. According to¹¹ in Kuwait determined that, in 1 of the 5 yoghurt samples, the presence of AFM1 (0.03µg/kg). Authors in¹² tested AFM1 in yoghurt in South Korea (Seoul) and it was found to be AFM1 detected 84-94% (ELISA) and 87-93% (HPLC) respectively. Some study showed that AFM1 concentrations were affected by the seasonal effect. They reported higher level of AFM1 in cold seasons compared to hot seasons. The reason is that in cold seasons milking animals are usually fed with compound feeds and thus concentration of AFB1 increases, which in turn increases AFM1 concentration in milk. So,

humidity affects the presence of AFB1 in feeds. *Aspergillus* spp. can easily grow in feedstuffs having humidity between 13% and 18%, and then they are able to produce aflatoxin in environmental humidity between 50% and 60%. For this reason, the level of AFM1 in feed in cool months is more than hot months¹³. In the present study, AFM1 was detected in 100% of the tested yoghurt samples by range from of 5-83ng/kg. Twenty samples (22.22%) had higher AFM1 level than the admissible level (50ng/kg) established the European Union. AFM1 in summer and autumn samples ranged from 5-80.5 and 5-83ng/kg.

4. Conclusion

Mycotoxins, when present at high levels in the diet, cause acute and/or chronic adverse health effects in animals and humans. These compounds may affect many target organs and systems, notably the liver, kidneys, nervous system, endocrine system, immune system, and blood. So, it is essential to have continuous monitoring program over milk and dairy products by governmental food inspection agencies. Beside this, keeping low AFB1 level in feed stuff is of importance.

5. References

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