

THE STUDY OF AFLATOXIN M1 IN MILK AND IN DAIRY PRODUCTS

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Abstract

Aflatoxins are mycotoxins of great interest to the dairy industry. Mycotoxins aflatoxins M1 can cause toxic effects called mycotoxicosis. It has been reported that mycotoxins are carcinogenic, tumorigenic and cause of skin diseases in a large number of organisms and cause hepatic carcinomas in humans. This study aims to identify the amount of M1 mycotoxins in dairy products used by consumers in the Republic of Kosovo. According to the results obtained from the analysis of processed milk samples, it's concluded that aflatoxin M1 content in all types of dairy products does not exceed the limits according to the applicable rule for food safety in the Republic of Kosovo.

Keywords: Aflatoxins, mycotoxins M1, carcinogenic, tumorigenic, mycotoxicosis

1. Introduction

Mycotoxins are secondary metabolites that can contaminate animal and human food. The primary metabolites of fungi as well as for other organisms are those compounds that are essential for growth. Mycotoxin's contamination of human and animal food remains a worldwide problem, according to FAO estimates up to 25% of food crops in the world are contaminated with mycotoxins. Mycotoxins are classified into several species ranging from Aflatoxin which is recognized as the most important group of mycotoxins, Alternaria toxins, Citrinina, Okratoxin, Patulin, Penicillic acid, Fumonizine, Sabutoxin. of animals. Mycotoxins can be found in various foods but are mostly found in meat, eggs and milk. *Aspergillus flavus* - The most significant mycotoxins produced by *Aspergillus* species include aflatoxins B1, B2, G1 and G2. Aflatoxins are mainly produced by *A. flavus*, *A. parasiticus*, and *A. nominus* and are highly potent carcinogens in humans and animals (Santin 2005). Aflatoxins are produced at temperatures of 12 ° C to 40 ° C (Koehler et al., 1985). Aflatoxins - are the most popular mycotoxins compared to mycotoxins Aflatoxins are composed of four groups such as B1, B2, G1, G2 and aflatoxins M1 and M2. Aflatoxins are also found in milk, cheese, corn, peanuts, nuts, almonds, figs, grape berries, spices, and a variety of other foods and sources. high carcinogen (Squire, 1981) and is usually the main aflatoxin produced by toxic strains. Milk contamination by Aflatoxin M1 results mainly from the conversion of aflatoxin B1 metabolized by enzymes mainly found in the liver. After the formation of aflatoxin M1 it is secreted in cow urine and milk. Aflatoxin B₁ in food is a

mycotoxin produced by *Aspergillus* molds that grow on wheat, especially in maize. Misery is a higher risk product worldwide because it is grown in the most contaminated climates with aflatoxins.

Mycotoxins are associated with various acute and chronic effects in humans and animals, depending on species susceptibility, gender, and age. Acute toxicity usually has a rapid onset and a marked toxic response that can result in some fatal cases, while chronic toxicity is characterized by exposure to a low dose over a long period of time, resulting in cancer and side effects. Other generally irreversible. Of course, the main consequences in human and animal health on mycotoxin exposure are related to chronic exposure (e.g., onset of cancer, renal toxicity, decreased immunity). However, the most common cases of mycotoxins are those of acute effects (e.g., turkey X syndrome, ergotism, etc.) (Bennett & Klich 2003).

Aflatoxins especially aflatoxin B₁ have high toxic and carcinogenic properties in humans and animals. The International Agency for Research on Cancer has classified aflatoxin B₁ as a group I carcinogen (International Agency for Research on Cancer, 1982). Aflatoxin has been linked to toxicity and carcinogenic diseases in both humans and animals (Eaton & Groopman, 1994). Diseases caused by aflatoxins can be termed as aflatoxicosis. Acute aflatoxicosis results in death of the organism, while chronic aflatoxicosis results in cancer, weakened immunity and other slow pathological conditions (Hsieh, 1988). The liver is the first organ to be affected, liver damage occurs when birds, fish, rodents and non-human primates are fed aflatoxin B₁. There are significant differences in species sensitivity. Furthermore, within the same species, response size is influenced by age, sex, weight, food diet, exposure to infectious agents, the presence of other mycotoxins, and pharmacologically active substances (Cullen & Newberne, 1994). Due to differences in aflatoxin sensitivity in the animals tested it has been difficult to assess the potential effects of aflatoxins in humans, but the acute toxicity of aflatoxins in *Homo sapiens* has not been observed very often. It is believed that in 1974 the hepatitis epidemic in India, in which 100 people died may have been caused by the consumption of corn, which was highly contaminated with aflatoxins. Some adults may have consumed 2 to 6 mg of aflatoxin a day (Krishnamachari et al., 1975). Subsequently, it has been estimated that the lethal dose for adults is 10 to 20 mg of aflatoxin consumed (Pitt, 2000).

2. Materials and methods

This paper, like any other paper, has its own characteristics, analyzes were conducted at the Kosovo Food and Veterinary Agency. First 20 samples were taken and analyzed starting from 3.5% UHT milk, 0.5% 1L sterilized milk, 3.8% milk, raw milk, chocolate milk, Yogurt, fresh milk, sterilized milk, 3.8% UHT milk, 3.5% milk and pasteurized milk. Samples were randomly sampled to ascertain the actual state of mycostoxins in milk and dairy products.

Used method: Aflatoxin M1 ELISA Test Kit Manual Cod.1060 - 09;

Used standards are :ISO 14675:2006, ISO 14675:2007, ISO 14675 :2008, ISO 14675 : 2009, ISO 14675:2010, ISO 14675 2011, ISO 14675:2012, ISO 14675 : 2013.

1. Working method - Take out 0.5 ml of the sample into a vial ,add 0.5ml of 1X Milk Extraction Buffer ,vortex for 3 minutes at maximum speed.

2. Use 200 μ L of the lower, aqueous layer for the assay (avoid contact with the top fat layer).

Elisa Testing Protocol

1. Add 200 μ L of each Aflatoxin M1 standard in duplicate into different wells.(Add standards to plate only in the order from low concentration to high concentration).
2. Add 200 μ L of each sample in duplicate into different sample wells.
3. Incubate the plate for 45 minutes at room temperature (20 – 25 °C)
4. Thoroughly decant or aspirate solution from wells and discard the liquid. Wash the plate 3 times with 250 μ L of 1X Wash Solution. After the last wash ,invert the plate and gently tap the plate dry on paper towels.
5. Add 100 μ L of Aflatoxin M1 – HRP Conjugate to each well.
6. Incubate the plate for 15 minutes at room temperature (20 – 25 °C)
7. Thoroughly decant or aspirate solution from wells and discard the liquid. Wash the plate 3 times with 250 μ L of 1X Wash Solution. After the last wash ,invert the plate and gently tap the plate dry on paper towels.
8. Add 100 μ L of TMB substrate. Time the reaction immediately after adding the substrate. Mix the solution by gently rocking the plate manually for 1 minute while incubating at room temperature.
9. After incubating for 15 minutes at room temperature (20 – 25 °C),add 100 μ L of Stop Buffer to stop the enzyme reaction.
10. Read the plate as soon as possible following the addition of Stop Buffer on a plate reader with 450 nm wavelength.

3. Results and discussion

In this study, 20 samples of dairy products were analyzed. These samples were analyzed for the purpose of detecting aflatoxin M1 in milk and its products. The following table shows that there is a higher number where Aflatoxin M1 is detected while a smaller number is not detected. The highest percentage of Aflatoxin M1 was detected 3.5% UHT milk with a result of $0.024\mu\text{g} / \text{kg} \pm 0.007\mu\text{g} / \text{kg}$, while the lowest percentage was detected fresh milk and UHT milk with a result of

$0.003 \pm 0.0009 \mu\text{g} / \text{kg}$. Whereas aflatoxin M1 is detected there are 2 different types of pasteurized milk and 2 other types of UHT milk.

In conclusion, out of 20 samples, 4 of them were not detected with Aflatoxin M1 and 16 of them were detected with a low percentage, within the limits set by regulations less than $0.05 \mu\text{g} / \text{kg}$.

Table 1. Results of the Aflatoxin M1 study of milk and milk improvement

No.	Type of sample	Used method	Standards, Reagents, Kits, Solutions	Result
1	UHT Milk 3.5%	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	$0.024 \mu\text{g}/\text{kg} \pm 0.007 \mu\text{g}/\text{kg}$
2	Sterilized milk 0.5% 1L	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	Not detected
3	Milk 3.8%	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	$0.016 \mu\text{g}/\text{kg} \pm 0.004 \mu\text{g}/\text{kg}$
4	Raw milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	$0.006 \pm 0.002 \mu\text{g}/\text{kg}$
5	Chocolate milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	$0.004 \pm 0.001 \mu\text{g}/\text{kg}$
6	Sirkos milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	$0.004 \pm 0.001 \mu\text{g}/\text{kg}$
7	Moja Kravica Yougurt	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	$0.014 \pm 0.004 \mu\text{g}/\text{kg}$
8	Fresh milk	Aflatoxin M1 Elisa Test Kit	ISO 14675:2006	$0.007 \pm 0.002 \mu\text{g}/\text{kg}$

		Manual Cod. 1060-09		
9	Sterilized milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	0.011±0.003µg/kg
10	Sterilized milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	0.018±0.005µg/kg
11	Fresh milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	0.003±0.0009µg/kg
12	UHT milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2006	0.003±0.0009µg/kg
13	UHT milk 3.5%	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-10	ISO 14675:2007	Have not been detected
14	UHTmilk 3.8%	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-09	ISO 14675:2008	0.031±0.009µg/kg
15	Milk 3.5%	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-10	ISO 14675:2009	Not detected
16	Pasteurized milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-11	ISO 14675:2010	0.017±0.009µg/kg
17	Pasteurized milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-12	ISO 14675:2011	0.015±0.004µg/kg
18	Pasteurized milk	Aflatoxin M1 Elisa Test Kit	ISO 14675:2012	Have not been detected

		Manual Cod. 1060-13		
19	Pasteurized milk	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-14	ISO 14675:2013	Have not been detected
20	UHTmilk 3.5%	Aflatoxin M1 Elisa Test Kit Manual Cod. 1060-10	ISO 14675:2007	Have not been detected

4. Conclusion

Referring to the results obtained from this paper we can conclude and recommend:

1. The products randomly sampled from the market meet the criteria of the applicable regulation for Aflatoxin M1.
2. In some products the presence of Aflatoxin M1 has not been detected.
3. In some products the presence of Aflatoxin M1 was detected but none of the samples exceeded the limit set in the 0.05 µg / kg regulation.
4. All the products analyzed in this study are safe for consumption by reference to local and European Union regulations.

Based on the data of this study and the data of various authors on this problematic we recommend:

5. Continuous monitoring of animal feed, as it is the first barrier where the problem with Aflatoxin M1 can be eliminated.
6. Continuous monitoring of fresh milk to prevent Aflatoxin M1 in dairy products.
7. Continuous monitoring of dairy products because it is a thermoresistant toxin and no technological process currently applied to foods can eliminate it.
8. Monitoring dairy products such as cheese that can increase Aflatoxin M1 concentration because a kilo of cheese requires 5 liters or more of milk depending on the type of cheese.

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