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Determination of Aflatoxin M1 in Pasteurized and Traditional Milk in Hamadan Province, Iran

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ABSTRACT

Introduction: Milk is one of the most complete food products that is effective in reducing blood pressure and increasing its beneficial fats, preventing colon cancer and osteoporosis, and providing many nutrients, such as protein and calcium. Therefore, the contamination of this valuable foodstuff and its products is considered as a serious risk to the public health of the community. Aflatoxin is a dangerous fungal toxin that is produced in the presence of moisture and heat as well as lack of proper storage conditions; moreover, it is considered as a hazardous substance in human health. The aim of this study was to evaluate the level of Aflatoxin M1 (AFM1) in raw milk, pasteurized and sterilized milk in food distribution centers of Hamadan province in 2016.

Materials and Methods: In the present study, 586 traditional and pasteurized milk samples (446 pasteurized milk samples and 140 traditional milk samples) produced in Hamadan province in summer of 2016 were investigated for AFM1 using Quick AFM1 Strip Test Code ASTM1/96 kit.

Results: AFM1 was not observed in 2% of traditional samples and 6.7% of pasteurized specimens. In this study, 37.85% of traditional milk, 56.3% of pasteurized samples had AFM1 less than 50 ppt. Moreover, 12.5% of traditional milk and 1.5% of pasteurized samples had AFM1 more than 50 ppt, which was higher than Iran standard limitation.

Conclusion: The results of this study indicate the presence of AFM1 toxin contamination in traditional and pasteurized milks of Hamedan province. Further investigation and monitoring is needed in Hamedan province.

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Introduction

Aflatoxins are natural fungal toxins and are mainly produced by special strains of Aspergillus flavus and Aspergillus parasiticus [1, 2]. These fungi are toxinogenic and contaminate food products at different stages of production, especially in appropriate moisture and heat conditions. Aflatoxins have several types, such as G2, G1 B2, and B1. Aspergillus flavus produces only aflatoxins B [3], while other species produce both

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aflatoxin B and G1. These toxins are found in numerous human and animal foods. Aflatoxicosis occurs in industrialized and advanced countries, and in addition to being dependent on environmental, social and economic conditions, it depends on climatic conditions such as humidity and heat that are suitable for fungal growth [4]. Aflatoxin M1 (AFM1) and Aflatoxin M2 (AFM2) are oxidative metabolites of Aflatoxin B1 (AFB1) and Aflatoxin B2 (AFB2), which are produced by the action of microsomal liver enzymes and are usually found in milk, urine and faeces of livestock, and some of the mammalian species that have been fed with aflatoxin contaminated food [4, 5]. AFM1 is bound to the protein of milk that is casein [6]. Aflatoxin is a combination of acute toxicity, immunosuppressive, mutagenic, teratogenic, and carcinogenic [7]. The World Health Organization (WHO) International Agency for Research on Cancer has identified AFB1 and AFM1 as the primary and secondary metabolites of carcinogens, respectively [8]. The target organ for toxicity and carcinogenicity is the liver. Although AFM1 is mutagenic and cassinogenic lower than AFB1, it has a higher genotoxic activity [9]. AFM1 resists the heat of pasteurization, autoclaving, and other stages of food process, and they are ineffective in reducing it [10, 11]. The European Codex Food and Drug Administration have set maximum levels of AFM1 in raw milk, powder, heat-treated milk and processed milk products at 50 ng/kg, which should not be exceeded [12]. According to the US regulations, the level of AFM1 should not exceed 500 ng/kg [13]. The Iranian Institute for Standardization has declared the limit of AFM1 in raw milk 0.5 µg/L [14]. In Australia and Switzerland, this figure has dropped to 10 ng/kg in children food [15]. The AFB1 contamination limit in the livestock feed is 5 µg/kg. Other mycotoxins may even exist in small amounts in milk and dairy products [16]. Since milk is an important nutrient in daily human diet, the aim of this study was to compare the AFM1 level in traditional and pasteurized milk in Hamadan province in the summer of 2016.

Materials and Methods

This cross-sectional study was performed on 586 traditional and pasteurized milk samples (446 and 140 traditional milk samples) with different consumption dates collected from supermarkets of Hamedan province during summer of 2016, in order to evaluate Aflatoxin. For testing AFM1, the Quick Afla M1 Strip Test Code ASTM1/96 kit, a fast and commercial test, was used, which its result is visible and readable by eyes. This kit is used to determine the amount of Aflatoxin remaining at 50-100 ppt. To carry out the test, first 200 µL samples from the milk sample were poured into a clear plastic reaction microwell. Then the lyophilized reagents were re-suspended to a uniform pink color in the bottom of the microwell for each sample. The milk containing pot was left 5 minutes so that the anti-Aflatoxin M1 is bonded with gold particles.

If the antibody linked to the gold particles has engaged with the AFM1 presented in the milk sample, the gold particles will flow past the T-Line and reach the Control line (C-Line). Figure 1 shows the visual interpretation of the test results.

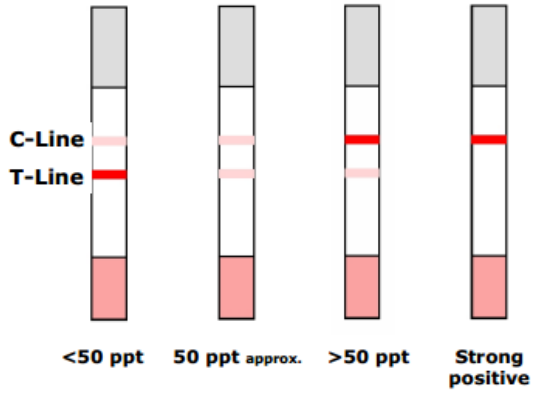

Figure 1: Interpretation of the results

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According to Figure 1 if the T-Line signal intensity is stronger than the signal at the C-Line it means that the result is lower than 50 ppt. If the signal at the T-Line is less intense compared to the C-Line it means that the concentration of AFM1 in the milk sample is higher than 50 ppt. If the two lines have equal signal intensities and it is not possible to distinguish which one is more intense by eye, then the test result is about 50 ppt. When only the C-Line is visible and the T-Line cannot be seen, the test result is strongly positive (equal or higher than 300-400 ppt).

Results

The contamination level of AFM1 pasteurized and traditional milk is presented in Table 1. Based on the results, AFM1 was not observed in 2% of the traditional samples and 6.7% of the pasteurized samples. In 47% of traditional milk and 35.5% of pasteurized samples, AFM1 was 50 ppt Moreover, in 12.5% of traditional milk and 1.5% of pasteurized samples AFM1 was higher than ppt 50 and in 37.85% of traditional milk and 56.3% of pasteurized samples AFM1was less than 50 ppt. Furthermore, the results showed that 95.7% of samples were according to Iran standards (Table 1, Figure 2).

Table 1: AFM1 levels in traditional and pasteurized milk in Hamadan province separated by cities

City		ND*	50 ppt	More than50 ppt	Less than 50 ppt
Bahar	Pasteurized	20	36	4	75
	Traditional	1	6	6	2
Hamedan	Pasteurized	5	33	3	108
	Traditional	0	11	0	33
Nahavand	Pasteurized	0	37	0	19
	Traditional	0	26	2	4
Malayer	Pasteurized	0	19	0	36
	Traditional	0	16	8	12
Toyserkan	Pasteurized	5	33	0	13
	Traditional	2	7	2	2
Total	Both	33	224	25	304

ND* = Not detected

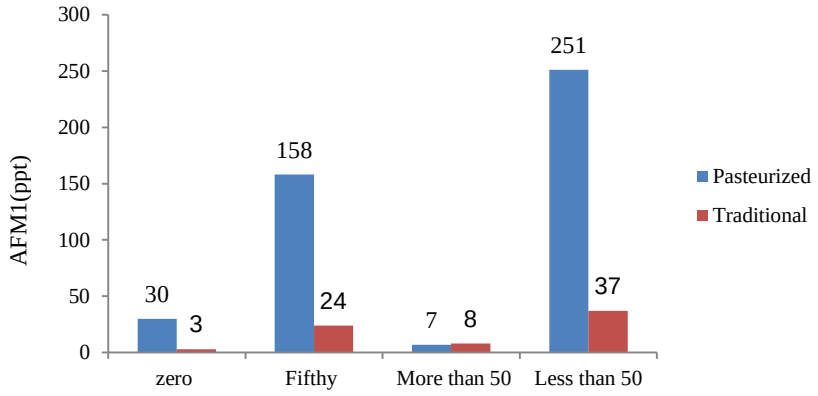

Figure 2: Characteristics of AFM1 in Traditional and Pasteurized Milk in Hamadan Province

Discussion

In this study, milk samples were collected and evaluated. The results showed that 95.7% of samples were in accordance with Iran standards. In general, it can be concluded that AFM1 infection in Hamadan province milk is not a serious health problem. Due to the fact that thermal processes such as sterilization and pasteurization have no effect on reducing the amount of milk aflatoxin [16], if the products in the province are provided from less polluted centers, they will be more consistent with international standards [16]. In a

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study conducted by Kim Yeak et al. in Korea, the prevalence of AFM1 in pasteurized milk and dairy products was investigated. Out of 180 samples collected from the capital of Seoul, 76% of pasteurized milk samples were contaminated with toxin with an average of 18 pg/g [17]. In the study of Golipour et al., which was performed on Mazandaran pasteurized milk, it was found that 96% of samples had Aflatoxin, and in 67.62% of the samples Aflatoxin concentration was higher than the limit established by European Codex Committee for Food and Diet [18].

In a study of 44 lactating dairy products in Taiwan in 2002, 90.9% of samples were contaminated with AFM1 [19- 20]. In a review study conducted by Sadeghi et al. in 2012, the results showed that Aflatoxin was higher than the standard in different milk and contamination was more in warm seasons than in cold seasons in most cases [16]. In the study conducted in Shiraz, the infection rate was reported in 100% of samples [21]. Although the necessity of supplementary studies in the province of Hamedan and provinces with contamination is recommended, preventive measures should be taken to prevent the entry of this toxin. Therefore, it is possible to reduce and control the amount of toxin by using the superior technology by experts and the application of rules and regulations in the promotion of the milk quality received by the community and animal feed.

Conclusion

The results of this study raise the issue of AFM1 in local and pasteurized milk, which showed a higher percentage of contamination in local sample than the pasteurized one. Although Aflatoxin is caused by poorly fed livestock by mildew breads and residues, milk of livestock should be tested and investigated for drug residues, vegetable pests and mycotoxins. Finally, it is suggested that the officials of the Agricultural Jihad and other organizations provide appropriate solutions to reduce the amount of contamination in the milk collection centers, such as control of animal feed and storage and keeping of forage and livestock feed. Therefore, careful and continuous monitoring of the control and maintenance of forage and animal feed in contaminated areas is necessary.

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Conflict of interest

The authors declare that there is no conflict of interest.

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