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Occurrence of Aflatoxin M_1 in Pasteurized and Local Yogurt in Mazandaran Province (Northern Iran) Using ELISA

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Abstract: Aflatoxis are fungi toxins which are incriminated for human carcinogenesis, immunosuppresion, tratogenesis and mutagenesis. In this study, 40 pasteurize yogurt samples and 10 local yogurt samples were collected from Mazandaran province (northern Iran) in autumn 2009 and were tested for Aflatoxin M_1 (AFM_1) contamination by competitive ELISA. It was found that 100% of pasteurizes yogurt samples and local yogurt samples were positive with concentrations of AFM_1 2.1-61.7 and 7-53 ng/l, respectively. Of 40 pasteurize yogurt samples 1 sample (2.5%) and of 10 local yogurt samples 1 sample (10%) contained AFM_1 with above the limit of European community regulations (50 ng/l) whereas 10% pasteurize yogurt samples and 30% local yogurt samples were contamination AFM_1 over than 25 ng/l (the standard limit for food children). Unsuitable storage of feedstuff after harvesting leads to mould growth and aflatoxin production. Since AFM_1 are not removed by pasteurize and heating, some measures are to be taken to prevent aflatoxigenic mould growth in feedstuff.

Key words: Aflatoxin M_1 · ELISA · Yogurt

INTRODUCTION

Aflatoxins are severe toxic secondary metabolites. Generally they are produced by special strains of *Aspergillus flavus, Aspergillus parasiticus* and *Aspergillus nomius*. Aflatoxins B_1, B_2, G_1, G_2 and M_1, M_2 (metabolites in milk) are dihydrofuran moiety metabolic or tetrahydrofuran which is bound to coumarin ring [1]. When animals consume feedstuff contaminated with AFB_1, the toxin is metabolized in liver and execrated as AFM_1 via milk, urination and feces [2]. These toxigenic fungi contaminate food products in different phases of production and processing, especially in suitable heat and moist conditions. Aflatoxicosis depends on both environmental, social and economical conditions and the climate [3]. AFM_1 and AFM_2 are oxidative metabolic AFB_1 and AFB_2 which are made by live mycosomal enzymes functions. AFM_1 and AFM_2 are discharged via milk, urinary and feces of cattle and some species of mammals that consume feed stuff contaminated with Aflatoxin [3, 4]. Their main target organ for toxicity and carcinogenicity is liver. Although mutagenic and carcinogenic level of AFM_1 is lower than AFB_1, its genotoxic activity is high. Aflatoxin in some human diseases, particularly, liver primary cancer is involved by engaging DNA, mutagen P_{53} gene [5]. AFM_1 appears in milk by cattle's consuming AFB_1 after 12-24 hours. Milk and dairy products consumption is high in human particularly among children; therefore, they are exposed to AFM_1. On the other hand, milk is consumed not only as liquid but as children formulae, yogurt, cheese and milk-based sweets such as chocolate and donuts. Therefore, AFM_1 recognition in milk and dairy products is crucial. As a result, consumers of various ages are protected from its potential risks [6]. European community and Codex Alimentary determined the maximum level of AFM_1 50 ng/kg in raw milk, liquid, dry, heat-treated milk and processed dairy products, cheese, yogurt and it must not be exceeded the amount [7]. AFM_1 resists against pasteurization and autoclave heat and other food producing and processing steps and there is no effect in decreasing them [2, 8]. Many countries have conducted inspection program and controlling on mycotoxins for several years to promote public health. *Gurby et al.* [9] in Ankara, Turkey in 2006 found AFM_1 in 22 yogurt samples out of 40, ranging from 61.61 to 365.64ng/kg. *Sarimehmetoglub et al.* [10] in 2002 collected 132 yogurt samples, to test their AFM_1 by ELISA. AFM_1 was found in 49 samples ranging from 50-800 ng/l. The aim of this study was to survey the occurrence of AFM_1 in Pasteurized and Local yogurt in Mazandaran province, northern Iran. There has been no detection in area.

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MATERIALS AND METHODS

Samples: A total of 50 yogurt samples, 40 pasteurize yogurt samples and 10 local yogurt samples) was randomly collected from Mazandaran province (northern Iran) in autumn 2009. Samples were tested for Aflatoxin M_1 (AFM_1) contamination by competitive Enzyme Linked Immunosorbent Assay (ELISA) by Tecna, AFLA M_1 kit. Solid phase in plastic micro wells was coated with anti-Aflatoxin M_1 antibodies.

Preparing: Yogurt samples were pasteurized in Ban Mari 80 centigrade for 30 minutes. Having cooled down in room temperature, they were diluted in buffer PBS (20 mM, pH:7.2) by 1:5 ratio. After mixing, 100 micro liter of the solution was used for ELISA.

Methods: We added 100 micro-liter samples extract and Aflatoxin M_1 standard solutions in each well. In each micro-plate, we appointed 7 wells for standards and then incubated the plate at 20-25 centigrade for 45 minutes. Each well was washed four times by washing buffer 20X concentration. Then 100 micro-liter conjugated solution (100X) was added to each well. After that, the plate was incubated at 20-25 centigrade for 15 minutes. Next, the wells were washed. Substrate then was added in the wells. Then we incubated the plate at 20-25 centigrade in a dark place for 15 minutes. The reaction was stopped by the stop solution. At most after one hour, light absorption was read at 450 nm by ELISA reader [9].

RESULTS

In this study, 50 yogurt samples were tested for aflatoxin M_1 (AFM_1) contamination by competitive ELISA. In 2.5% of pasteurize yogurt samples the presence of AFM_1 over than concentration 50 ng/l and 10% local yogurt samples was over than concentration 50 ng/l (limit of European community regulations) whereas, 10 and 30% were detected by concentration above 25 ng/l (standard limit for food children), respectively. The AFM_1 contamination levels in pasteurize yogurt local yogurt samples were between 2.1-61.7 ng/l by the average concentration of 15.13 and 7-53 ng/l with mean of 24.6 ng/l, respectively (Tables 1, 2). Data were analyzed by ANOVA utilizing SPSS software package. The results were analyzed by comparing them with standard limits. There was a significant relationship between AFM_1 contamination level and kind yogurt applying statistical test.

Table 1: The AFM_1 level distribution in autumn 2009 in pasteurizes and local yogurt samples from Mazandaran province, northern Iran.

		Percent positive sample					
Kind yogurt	Number samples	>25 ng/l	>50 ng/l	Mean±SE	S.D.	Max	Min
pasteurize	40	10	2.5	15.13±1.75	11.04	61.7	2.1
local	10	30	10	24.6±5.08	16.08	53	7
Total	50	40	12.5	17.02±1.78	12.62	61.7	2.1

S.E= Standard Deviation, S.D=Standard Error of Mean, ng/l=nanogram/liter

Table 2: The distribution of AFM_1 level concentration in pasteurized and local yogurt samples from Mazandaran province, northern Iran.

	Frequency distribution of sample (ng/l)							
	<9		10-25		26-49		>50	
Kind yogurt	N	%	N	%	N	%	N	%
pasteurize	15	37.5	21	52.5	3	7.5	1	2.5
local	1	10	6	60	2	20	1	10
Sum	16	32	27	54	5	10	2	4

N=Number

Table 3: AFM_1 contamination in yogurt in different countries

Country	Year	Number	%(> 50ng/l)	Researchers	Reference
Turkey	2006	177	38.65	Akkaya	11
Turkey	2006	40	58	Gurby	9
Italy	1998	114	80	Galvano	15
Italy	2001	120	61	Galvano	12
Portugal	2004	96	34	Martins	13
South Korea	2000	60	50	Kim	14

DISCUSSIONS

As epidemiological studies in Iran on AFM_1 contamination in milk and dairy products have less been conducted, then it is essential to be placed on the government agenda. Doing such regulations actually will lead to reduce contamination to aflatoxigenic moulds in feed stuff. *Akkaya et al.* [11] in Turkey, in 2006 177 yogurt samples (104 ordinary yogurts, 21 fruit yogurts and 52 Torba yogurts) were tested for AFM_1 by ELISA. The highest AFM_1 contamination was found in Torba yogurt (150 ng/kg) and in the ordinary yogurt was 100 ng/kg and fruit yogurt contained normal concentration. The contamination in ordinary yogurt was 65.38% in fruit yogurt 33.33% and Torba yogurt 55.77%. Moreover, 11.53% of ordinary yogurt, 9.52% fruit yogurt and 21.15 % Torba yogurt contained AFM_1 higher than, 50 ng/kg. *Gurby et al.* [9] in Turkey found AFM_1 in 22 yogurt samples out of 40, ranging from 61.61 to 365.64 ng/kg. *Galvano et al.* [12] in 2001 randomly collected 120 yogurt samples at supermarkets in four large cities in Italy. AFM_1 was found in 73 samples ranged between one and 3.1 ng/kg. Whereas our study The AFM_1 contamination levels in pasteurize yogurt local yogurt samples were between 2.1 - 61.7 ng/l and by the average concentration of 15.13 ng/l - 24.6 ng/l, respectively. *Martins et al.* [13] in 2004, 96 local yogurt samples (48 natural yogurt and 48 with pieces of strawberry) were tested by HPLC in Portugal. AFM_1 was found in 18 samples ranging from 19 to 98 ng/kg and in 78 samples, AFM_1 was not found. 2 out of natural yogurt were contaminated with AFM_1 by 43 and 45 ng/kg. Of 48 yogurt samples with pieces of strawberry, 16 ones contained contamination ranging from 19 to 98 ng/kg. *Sarimehmetoglub et al.* [10] in 2002 collected 132 yogurt samples, to test their AFM_1 by ELISA. AFM_1 was found in 49 samples ranging from 50-800 ng/l. whereas our study 2.5% of pasteurize yogurt samples and 10% local yogurt samples was over than concentration 50 ng/l which 10% and 30% were detected by concentration above 25 ng/l, respectively. *Kim et al.* [14] tested AFM_1 by ELISA and HPLC in yogurt in Seoul, South Korea. AFM_1 was detected 84-94% and 87-93, respectively. The limit detection by ELISA and HPLC was 2 and 10 pg/ml, respectively. The results obtained by ELISA and HPLC were similar.

To decrease AFM_1 in milk to the lowest point, feed stuff ration should be checked regularly and it should be kept away from fungal contamination. To increase milk quality, it is necessary for feed stuff to be without AFB_1 contamination [14]. In northern Iran due to favorable aflatoxigenic moulds growth, some measures should be taken in production, processing and storage of feed stuff. Because *Gholampour Azizi et al.* [16] in Babol city (northern Iran) detected AFM_1 in 100% milk to the concentration 193-259 ng/l and *Hadizadeh et al.* [17] in northern Iran were found aflatoxin B_1 in animal food between 10.4 to 68.8%. Moreover *Gholampour Azizi et al.* [18] in same area detected mycotoxin of the Zeralenone and Ochratoxin in human food 5-8.3 and 5-7.5%, respectively. It is a serious public health problem, because all age groups including babies and children extensively consume the product. As a result, milk and dairy products should be controlled regularly at least twice a year. Beside this, keeping low AFB_1 level in feed stuff is of importance. To reach the goal, feed stuff should be kept away from probable contamination. Milk and dairy products are main human food, especially for children because they are more sensitive to Aflatoxin effects and their ability to biotransformation of these carcinogens agents is generally slower than adults. In any case, the products may be contaminated and are risky to humans. As a result, most countries have some regulations to control AFB_1 in feedstuff and permitted amount of AFM_1 in milk to reduce risks.

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