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Aflatoxin M1 in Yoghurts, Cheese and Ice-Cream in Shahrekord-Iran

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Abstract: Aflatoxin M_1 (AFM_1) may occur in milk products which is the hydroxylated metabolite of aflatoxin B_1 in feedstuffs of dairy cow. 120 samples of yoghurts, cheese and ice-cream (40 each) that are produced in Iran were analyzed for AFM_1 contamination by an ELISA kit. AFM_1 was detected in 35, 40 and 29% of yogurt, cheese and ice-cream samples, respectively. The concentration of AFM_1 in 7 samples of cheese was higher than the standard limit (<250 $ng.l^{-1}$), but yoghurt and ice-cream samples had lower concentrations than the maximum tolerance accepted.

Key words: Aflatoxin M1 · Milk Products · ELISA · Iran

INTRODUCTION

Aflatoxins can be produced from fungal metabolites in a wide range of important commodities. Aflatoxin M_1 (AFM_1) is the hydroxylated metabolite of aflatoxin B_1 (AFB_1) that it can be found in milk and dairy products. When lactating mammals, such as cows, sheep and goats are fed with feedstuffs containing AFB_1, this metabolite can be converted to AFM_1, which is cytotoxic and genotoxic [1]. About 1-2% of AFB_1 in animal feed is transformed to AFM_1 in milk with variations from animal to animal, from day to day and from one milking to the next and when the intake of AFB_1 is stopped, the AFM_1 concentration in the milk decreases to an undetectable level after 72 h [2, 3].

In the assessment of cancer risk, the infants are more exposed to the risk because the milk is a major constituent of their diet. Therefore the presence of AFM_1 in milk and milk products is undesirable [2, 4] and a particular risk for human. So the purpose of this survey was to determine the levels of AFM_1 in samples of yoghurt, cheese and ice-cream manufactured in Iran using locally produced milk, during 2011.

MATERIALS AND METHODS

Samples: A total of 120 samples of yoghurt, cheese and ice-cream (traditional and industrial) was collected from different supermarkets in Shahrekord, Iran. Two gm from each sample were analyzed after thoroughly mixing the product.

Aflatoxin M1 Determination and Quantification: The analysis of AFM_1 was performed using Ridascreen aflatoxin M1 kit (R- Bipharm AG, Germany). Reading was performed at a wavelength 450 nm in an ELISA reader as modification of Ozaslan *et al.* [5]. Light absorption is inversely proportional to the AFM_1 concentration in the sample [6].

RESULTS AND DISCUSSION

The incidence of AFM_1 contamination in yoghurt, cheese and ice-cream samples was 35, 40 and 29%, respectively. Only 7 samples of cheese showed contamination higher than European Commission limit (> 250 ng/kg) [7]. Forty yoghurt and ice-cream samples did not reveal the presence of toxin (< 10 ng/kg). The mean of AFM_1 in ice-cream was lower than yoghurt and cheese (Table 1). In Brazil, Sylos *et al.* [8] did not detect the presence of AFM1 in 30 samples of the yoghurt because cows graze all year round. According Galvano *et al.* [9] study results, 80% of samples of yoghurt were contaminated with levels of AFM_1 ranging from 1-496.47 ng/kg. In other research, from 120 yoghurt samples, 61% were contaminated with AFM_1 at lower levels

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Table 1: Occurrence of aflatoxin M_1 (ng/l) in the examined yoghurt, cheese and ice- cream samples

	Aflatoxin M_1				
Type of dairy product	Tested samples	Positive samples(%)	Mean	Minimum	Maximum
Yoghurt	40	35(%)	130.5	11.4	115.8
Cheese	40	40(%)	133.2	31.9	505.7
Ice-cream	40	29(%)	65.1	20.1	197.4
Total	120	104(%)	328.8	11.4	505.7

(1-32.1 ng/kg) [10]. Another research reported that the mean concentrations observed in pasteurized milk samples were 39 $ng.l^{-1}$ [11]. Increases of AFM_1 content in dairy products can relate to this contamination in milk [12]. The decrease in AFM_1 can be attributed to factors such as low pH, formation of organic acids or even to the presence of lactic acid bacteria. The low pH could alter the structure of milk proteins and leading to formation of yoghurt coagulum that it may affect the association of AFM_1 with this protein [13] causing adsorption of the toxin. Also enzymatic, microbial and particularly acid coagulation can cause degradation of AFM_1 in milk [14]. According to other results, AFM_1 is more stable in the yoghurts with pH 4.6 than pH 4.0 during refrigerated storage [15]. The occurrence of aflatoxin in strong acid can cause its acid-catalyzed hydration [16], but not its degradation or neutralization. The high levels of AFM_1 contamination in milk were traced to the cattle feed-corn that was harvested in the summer and extremely hot temperatures and high humidity favored the development of AFB_1-producing molds during the storage corn crops [17].

In other research, 80% of cheese samples were contaminated with AFM_1 in a range 23.8-452 ng/kg, with one sample being above the regulatory limit (250 ng/kg) [18]. Investigations of several authors reported increases in AFM_1 concentration in cheese as a function of cheese type, technologies and the amount of water eliminated during processing [12, 19, 20]. Some factors such as renneting temperature, press time and saturated brine pH can affect the amount content of AFM_1 in the cheese curds [21]. Additionally, other studies showed that the amount of AFM_1 in white pickled and Kashar cheeses did not significantly affect these cheeses over the storage [20, 22] and the final ripened cheese was free of AFM_1 [23]. These different profiles of AFM_1 in various cheese products may be the result of several factors such as heat treatment, proteolysis and exposure of contaminated milk to light [24]. One study indicated that there is no simple physical method to remove AFM_1 from ovine and caprine milk [25]. Neither ultra filtration, nor acidic or enzymatic treatments were able to influence the toxin's interaction with casein or whey proteins. Only the combined action of heat and low pH are able to denature whey proteins where they lost their AFM_1-binding capacity. Other factors such as extraction techniques, methodology, type and degree of milk contamination, differences in milk quality and the presence of a small portion of curd in whey also can influence AFM_1 concentration and the cheese manufacture process [26]. The occurrence of AFM_1 was studied in Portuguese soft cheese, that in a total of 42 samples no one was contaminated with AFM_1 [27]. The occurrence of AFM_1 in cheese is rather infrequent except when AFB_1 is found in feeds for dairy cattle [28]. In Babol City in general, of 45 samples ice- cream, 22.2% were positive with above the limit of European community regulations (50 ng/l) [29]. AFM_1 incidence in samples can be sourced from the feeds of animals from which milk is got become contaminated with aflatoxin or *Aspergillus spp* [30].

As a result, the transformation from AFB_1 into AFM_1 in milk depends on factors such as the animal's age, immunological profile, bloodline, breed and health. The milk comes from dairy cows that live in better conditions in terms of both health and hygiene, especially regarding the animals' udders so organic milk is surely less contaminated than milk from sources using conventional production methods [17]. So it is important to control dairy products and animal feed for presence of aflatoxins and hygienic managements. Because of consumption of contaminated feeds by dairy animals and formation of AFM_1 in milk, the easiest way to deal with this problem is reducing the AFB_1 concentration in animal feed by improved processing and storage practices that control the feeds given to dairy animals and cause the reduction of AFB_1 amount to low levels.

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