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Short Communication

Determination of aflatoxin M1 in raw milk and traditional cheeses retailed in Egyptian markets

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AFM1 (Aflatoxin M1) is the major mycotoxin frequently found in milk and dairy products. A total number of 50 raw milk samples and 150 cheese samples (fifty of soft cheese, hard cheese and processed cheese samples) were analyzed. All of the samples were randomly purchased from Egyptian supermarkets in Alexandria city from February 2008 to March 2009. The method used was ELISA. Results showed that the mean concentration values of AFM1 in soft and hard cheese samples were higher than that in raw milk samples. Processed cheese samples were the least contaminated samples. All positive samples of raw milk and cheeses are exceeding Egyptian regulations (free from AFM1) while all of them are within the US regulations (500 ng/l or Kg). All positive samples of cheese were exceeding the European Commission regulation (50 ng/l or Kg), while 52.6% of examined raw milk samples were exceeding the European Commission regulation.

Key words: Aflatoxins M1, raw milk, cheese, ELISA.

INTRODUCTION

Milk and dairy products are fundamental components in the human diet, and may be the principle way for entrance of aflatoxins into the human body (Galvano et al., 1998).

Aflatoxins are toxic metabolites, generally produced by *Aspergillus flavus, A. parasiticus* and *A. nomius* (Creppy, 2002). They have immunosuppressive, mutagenic, teratogenic and carcinogenic effects, especially on the liver (Baskaya et al., 2006).

Aflatoxin M1 (AFM1) is a hydroxylated metabolite of aflatoxin B1 and can be detected in milk and dairy products from dairy cattle that have ingested feed contaminated with aflatoxin B1. Its parent molecule has been categorized as Class 1 human carcinogen (IARC, 1993), while AFM1 has a carcinogenity of 2 - 10% (Zinedine et al., 2007). The result of their toxicity ranged from gastroenteritis to cancer. The presence of Mycotoxins in food and feed depends on many biological factors, such as region, season, humidity and temperature, as well as the conditions under which crops are harvested, stored and processed. The presence of AFM1 in milk and milk products is considered undesirable due to toxic and carcinogenic properties (Prado et al., 2000).

Regulatory limits throughout the world are influenced by economic considerations and may vary from one country to another (Stoloff et al., 1991; Van Egmond, 1989). The European Community and Codex Alimentarius has prescribed that the maximum limit of AFM1 in liquid milk and dried or processed milk products is 50 ng/kg (Codex Alimentarius Commission, 2001; European Commission Regulation, 2001). According to USA regulations, the level of AFM1 in milk should not be higher than 500 ng/kg (Stoloff et al., 1991). In Egypt, the Ministry of Health established that fluid milk and dairy products should be free from AFM1 (Egyptian Regulations, 1990). Although the mycological quality of raw milk and cheese in Egypt has been studied extensively, there are rare available data on the content of AFM1 in raw milk and cheese.

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Table 1. Concentrations of AFM_1 in raw cow milk and cheese samples.

Sample category	Samples analyzed	Positive samples		AFM_1Concentration (ng/l or Kg)	
		No.	(%)	Range	Mean ± SD
Raw cow milk	50	19	38	23 - 73	49.74 ± 17.26
Soft cheese	50	20	40	52 - 87.6	70.63 ± 18.42
Hard cheese	50	19	38	51.6 - 182	132.24 ± 57.48
Processed cheese	50	11	22	51.8 - 54	52.52 ± 13.56

Table 2. Levels of AFM_1 (ng/l or Kg) in raw milk and cheese samples exceeding limits established by the EC/Codex, Egyptian and US regulations.

Sample category	Positive samples	Exceeding EC regulations		Exceeding Egyptian regulation		Exceeding US regulation	
		No. (%)	Range	No. (%)	Range	No. (%)	Range
Raw cow milk	19	10 (52.6)	51.8-73	19 (100)	23 - 73	-	-
Soft cheese	20	20 (100)	52 -87.6	20(100)	52 - 87.6	-	-
Hard cheese	19	19(100)	51.6-182	19 (100)	51.6 - 182	-	-
Processed cheese	11	11 (100)	51.8-54	11 (100)	51.8 - 54	-	-

The aim of this study was to investigate AFM1 levels in raw milk and cheese samples (soft, hard and processed cheese) retailed in Alexandria city in Egypt, to compare these levels with maximum AFM1 limits adopted by European, USA, and Egyptian regulations and to determine the potential risk posed to human health by the consumption of these products.

MATERIALS AND METHODS

Sample collection

A total number of 50 raw milk samples and 150 cheese samples (fifty of soft cheese, hard cheese and processed cheese samples) were analyzed for AFM1. All of the samples were randomly purchased from Egyptian supermarkets in Alexandria city from February 2008 to March 2009. All samples were stored 3°C in chillers prior to analysis.

Methods and analysis

The method used in this study was the enzyme-linked immunosorbent assay (ELISA). Commercial ELISA kits were purchased from Biopharm: RIDAScreen aflatoxin M1 (Cat. No. R1101) and RIDAScreen aflatoxin total (Cat. No. R4701). The detection limit for milk samples were 5 and 50 ng/kg for cheese, with recovery rates of 95% and 102 % for milk and cheese, respectively. All milk and cheese samples were prepared and defattened using the method outlined in the ELISA kits, as briefly described.

Raw milk samples

Twenty milliliters (20 ml) of liquid milk was centrifuged at 3500 g/10°C. The fatty layer was removed, and 100 µl of the defatted milk was applied directly in the ELISA kit for AFM1 determination.

Cheese samples

Cheese samples were prepared according to the method outlined in the ELISA kit. Two grams of a representative cheese samples were added to 40 ml of dichloromethane. The mixture was extracted by shaking for 15 min. Suspension was filtered and 10 ml of the filtrate were evaporated at 60°C under weak N_2 stream. The oily residue was redissolved in 0.5 ml methanol, 0.5 ml phosphate buffer saline and 1 ml of heptane. The mixture was centrifuged at 2700 g/15°C for 15 min. The upper layer of heptane was removed and 100 µl of the aliquot were diluted with 400 µl of kit buffer. 100 µl of the diluted samples were applied in the kit. The statistical software package SPSS version 16 was employed.

RESULTS

As it is shown in Table 1, the mean concentration values of AFM1 in soft and hard cheese samples were higher than that in raw milk samples. Processed cheese samples were the least contaminated type of cheese.

Table 2 shows that all positive samples of raw milk and cheeses are exceeding the Egyptian regulations, while all of them are within the US regulations (500 ng/l or Kg). All positive samples of cheese were exceeding the European Commission regulation (50 ng/l or Kg), while 52.6% of examined raw milk samples were exceeding European Commission regulation.

DISCUSSION

Milk and other dairy products are always at risk of being contaminated with aflatoxin M1. Parallel to the increasing amount of milk and dairy product consumption, studies on the presence of AFM1 in cheese products have been increasing globally as well as in Egypt.

Occurrence of AFM1 in cheese can be due to three possible causes: (1) AFM1 present in raw milk because of carryovers of AFB1 from contaminated cow feed to milk, (2) Synthesis of AF (B1, B2, G1 and G2) by *A. flavus* and *A. parasiticus* growing on cheese (Zerfiridis, 1985), and (3) Occurrence of these toxins in dried milk used to enrich the milk which is being used in the production of cheese (Blanco et al., 1988). However, the increase in AFM1 concentration in cheese has been explained by the affinity of AFM1 for casein (Allcroft and Carnaghan, 1963; Grant and Carlson, 1971; Applebaum et al., 1982; Brackett and Marth, 1982).

The wide range of AFM1 in hard cheese can be ascribed to different factors such as extraction technique, methodology, type and degree of milk contamination, differences in milk quality, expression of the results, the presence of a small portion of curd in whey which could influence AFM1 concentration, and the cheese manufacturing process (Blanco et al., 1988).

Lower incidence of AFM1 (3.3%) in soft cheese was obtained by Abou-Zeid et al. (1996) and Higher AFM1 concentrations in soft cheese (2.61µg/kg), hard cheese (4.5 µg/kg) and processed cheese (5.73 µg/kg) was reported by El-Sherief (2000).

Since AFM1 is a metabolite of AFB1 excreted in milk, detecting highly increased concentrations of AFM1 in cheese samples implies the presence of very high AFB1 levels in feed. Alexandria city is located in the north part of Egypt with very harsh winter seasons. Farmers in this part of Egypt harvest hay in the summer and feed it to the cattle during the winter. Fungi present in haystacks may easily produce toxins in inappropriate storage conditions. Following the consumption of highly contaminated feed with AFB1, conversion of AFB1 to AFM1 takes place in the liver and leads to elevated levels of AFM1 in the milk. Therefore, it is important to reduce the occurrence of toxins (AFB1) in feedstuff and take prophylactic measures to prevent factors enhancing toxin production. These factors include environmental temperature, humidity, and moisture content of the feed as well as pH and mechanical damage to the grain affecting mold production. Hence, management practices in harvest and storage regarding the aforementioned factors could decrease AF occurrence in feed. These results suggest that it is important to prevent toxin production in these products from the production stage to consumption as well as creating effective detoxification processes

Conclusion

Frequent analytical surveillance by food control agencies is highly recommended to control the incidence of mycotoxin contamination in Egypt especially in dairy products. Implementing a food control system, such as the HACCP system in the food industries, suggest an efficient means for limiting mycotoxin contamination in the Egypt's food supply.

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