

AFLATOXIN M1 LEVELS IN MILK AND SOME DAIRY PRODUCTS IN ALEXANDRIA CITY

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ABSTRACT

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Aflatoxin M1 (AFM1) is the major mycotoxin frequently found in milk and dairy products. AFM1 is a carcinogenic metabolite often found in dairy products. The present study was carried out to evaluate the occurrence of AFM1 in milk and some dairy products in Alexandria city. A total number of 150 samples, 30 each of Raw milk, Kareish cheese, Damietta cheese, Ras cheese and Yoghurt were randomly purchased from supermarkets in Alexandria city from January to August 2013 for determination of AFM1 by using direct competitive enzyme-linked immunosorbent assay (ELISA). Results showed that the mean concentration values of AFM1 was detected in 40 % of the analyzed milk samples with levels ranging from 8.30 to 85.00 ng/kg, AFM1 was detected in 46, 53 and 56 % of examined Kareish cheese, Damietta cheese and Ras cheese samples with levels ranging from 6.20 to 70.26, 16.50 to 133.2 and 7.40 to 111.50 ng/kg, respectively. While, results showed that AFM1 was detected in 26 % of analyzed yoghurt samples with level ranging from 11.40 to 98.80 ng/kg. All positive samples of raw milk, cheeses and yoghurt are exceeding Egyptian regulations (1990), while all of them are within the US regulations (Stoloff *et al.*, 1991). While 71, 75, 88%, of positive samples in kareish cheese, Damietta cheese and Ras cheese samples, respectively were exceeding the European Commission Regulation (Codex Alimentarius Commission, 2001). Also 58 % of positive raw milk samples and 63% of positive examined yoghurt samples were exceeding the European Commission regulation. It was concluded that widespread occurrence of AFM1 in raw milk and some dairy products samples were considered to be possible hazards for public health especially children.

Key words: Aflatoxins M1, raw milk, dairy products, 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). The consumption of contaminated milk and dairy products especially cheese is an important risk factor for consumers in particular for infants and young children, (Colak, 2007).

Aflatoxins are toxic metabolites, generally produced by *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (Creppy, 2002). 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. (IARC, 1993). Both AFM1 and AFB1 can cause DNA damage, gene mutation, chromosomal anomalies and cell transformation in mammalian cells in vitro (Govaris, *et al.*, 2002). The occurrence of AFM1 in commercially available milk and dairy products is a matter of interest for public health due to continuous and prolonged exposure to a carcinogenic substance. Aflatoxin M₁ presence in milk is considered as a potential risk for human health because of its

carcinogenicity potential and thus a need of regular monitoring in milk and dairy products (Prado *et al.*, 2000). The presence of AFM1 in cheese is thus considered undesirable due to toxic and carcinogenic properties (Amer and Ibrahim, 2010).

In addition, several authors have demonstrated that AFM1 is bound to milk proteins (Prandini *et al.*, 2009), mainly casein, and therefore the toxin is more concentrated in cheese than in the milk used to produce it.

Kareish cheese is one of the most popular fresh skim milk soft cheeses in Egypt. The increasing demand by Egyptian consumers is mainly attributed to its high protein content and low price (Osman *et al.*, 2012). AFM1 in Kareish cheese samples during storage might be attributed to the affinity of AFM1 for caseins which may cause concentration effect during milk process as well as the rather stable pH values (Capei and Neri, 2007).

AFM1 resistant to heat treatment and mild acidic conditions used in the production of cheese or other dairy products (such as, yoghurt) has been accounted for the contamination of such products (Colak, 2007 and Oruc *et al.*, 2007).

Regulatory limits throughout the world are influenced by economic considerations and may vary from one country to another (Stoloff *et al.*, 1991). The European Community and Codex Alimentarius have prescribed that the maximum limit of AFM1 in liquid milk and milk products is 50 ng/kg (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 dairy products. The occurrence of AFM1 in commercially available milk and dairy products is a matter of interest for public health due to continuous and prolonged exposure to a carcinogenic substance. As the ingestion of contaminated food is the primary route of potential human exposure to aflatoxins, the aim of this research is to determine the concentrations of AFM1 in raw milk and some dairy products retailed in Alexandria city, to compare these levels with maximum AFM1 limits adopted by Egyptian, European and USA regulations and to determine the potential risk posed to human health by the consumption of these products.

MATERIALS and METHODS

Samples:

A total number of 150 samples 30 from each of raw milk, Kareish cheese, Damietta cheese, Ras cheese and yoghurt were randomly obtained from super markets in Alexandria city. Samples were transported to the laboratory in an insulated container at about 4°C and analyzed for AFM1.

Sample preparation:

All milk and dairy products samples were prepared using the method outlined in the ELISA kits and according to official method specified in ELISA-System Aflatoxin M1. (RIDAScreen aflatoxin M1 (Cat. No. R1101).

- Raw milk samples

Twenty milliliters of liquid raw milk was centrifuged at 3500 rpm/10 minutes. 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 homogenized then added to 40 ml of dichloromethane. The mixture was extracted by shaking for 15 min., the suspensions were filtered and 10 ml of the filtrate were evaporated at 60°C under weak N₂ stream. The oily residue was re-dissolved in a mixture of 0.5 ml methanol, 0.5 ml

phosphate buffer saline and 1 ml of heptane. The mixture was centrifuged at 2700 rpm/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.

- Yoghurt samples

Ten grams of yoghurt samples were added to 100 ml of warm (20-25°C) deionized water and shaken for 10 min in speed of 250 min⁻¹. Subsequently, these samples were centrifuged at 3500 rpm/ 10 min at 4°C. The upper creamy layers were completely discarded and the lower phases were further diluted 20 times (v/v) with deionized water and then were used for the quantitative test according to the method outlined in the ELISA kit.

ELISA test procedure:

Analysis of AFM1 in samples by competitive ELISA

The quantitative analysis of AFM1 in milk and dairy products samples was performed by competitive ELISA (RIDASCREEN AFM1, R-Biopharm) procedure as described by R-biopharm GmbH (1999). Which is a competitive enzyme immunoassay based on antigen-antibody reaction. The wells in the micro-titer strips were coated with specific antibodies to AFM1. 100 µl of sample solution + 100 µl of standard 10 ng/kg (standard addition method was used because of detection limit 5 ng/kg) were added to the wells to occupy the binding sites proportionately, then mixed gently and incubated for 60 min at room temperature in the dark. Then the liquid samples were poured out of the wells and the wells were filled with 250 µl distilled water and poured out the liquid. Further steps were done according to the kit instruction and ultimately each well was washed four times by washing buffer. After almost one hour, light absorption was read at 450 nm by ELISA reader. The standard curve was used for determination related to the kit, milk and dairy products samples by competitive ELISA.

RESULTS

As it is shown in **Table 1** the mean concentration values of AFM1 in Damietta cheese, Ras cheese samples were higher than that in raw milk samples, while the mean concentration values of AFM1 in Kareish cheese samples were lower than that of raw milk samples, Yoghurt samples were the least contaminated type of examined dairy products.

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). 71, 75, 88, 63% of positive samples in Kareish cheese, Damietta cheese, Ras cheese and Yoghurt samples were exceeding the European Commission regulation (50 ng/l or Kg), while 58 % of examined raw milk samples were exceeding European Commission regulation.

Table 1: Concentrations of AFM1 in raw milk and some dairy products Samples.

Sample category	Samples analyzed	Positive samples		AFM1 Concentration (ng/l or Kg)		
		No	(%)	Minimum	Maximum	Mean \pm SD
Raw milk	30	12	40	8.30	85.00	32.56 \pm 5.98
Kareish cheese	30	14	46	6.20	70.26	28.66 \pm 4.66
Damietta cheese	30	16	53	16.50	133.2	53.059 \pm 8.013
Ras cheese	30	17	56	7.40	111.50	56.048 \pm 6.29
Yoghurt	30	8	26	11.40	98.80	28.41 \pm 4.78

Table 2: Levels of AFM1 (ng/l or Kg) in samples exceeding limits established by the Egyptian regulations and EC/Codex.

Sample category	Positive samples		Exceeding Egyptian Regulation (free from AFM1)		Exceeding EC regulations (50 ng/ Kg)	
	No.	(%)	No	(%)	No.	(%)
Raw milk	12	40	12	100	7	58
Kareish cheese	14	46	14	100	10	71
Damietta cheese	16	53	16	100	12	75
Ras cheese	17	56	17	100	15	88
Yoghurt	8	26	8	100	5	63

DISCUSSION

Food safety and foodborne diseases constitute a growing public health problem (WHO, 2009). Thus, great attention must be given to food control especially mycotoxins investigation in food due to their deleterious effect on human health. Mycotoxins are metabolites of fungi capable of having acute toxic, carcinogenic, mutagenic, teratogenic, immunotoxic, and oestrogenic effects in man and animals (Van Egmond *et al.*, 2007). Aflatoxin M1 (AFM1) particularly is the principal hydroxylated metabolite of aflatoxin B1 excreted in milk, and subsequently it can be found in a large variety of dairy products such as cheese thus posing a potential

risk to human health when consuming these products (Barug *et al.*, 2004).

The quality of milk products has a strong influence on the health of people in various age brackets. AFM1 is known to be hepatotoxic and carcinogenic. The World Health Organization changed its classification from group 2 to group 1 (Creppy, 2002). In this study the mean concentration values of AFM1 was detected in 40 % of the analyzed milk samples with levels ranging from 8.30 to 85.00 ng/kg, AFM1 was detected in 46, 53 and 56 % of the examined Kareish cheese, Damietta cheese, Ras cheese samples with levels ranging from 6.20 to 70.26 ng/kg, 16.50 to 133.2 ng/kg and 7.40 to 111.50 ng/kg, respectively.

Furthermore, high incidence similar to our results was reported in North Africa. Forty-nine samples of raw cow's milk were collected directly from 20 dairy factories in the north-west of Libya and analyzed for the presence of AFM1. Thirty-five milk samples (71.4%) showed AFM1 levels between 0.03 and 3.13 ng/ml (Elgerbi, 2004).

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). Aflatoxins may be found in cheese originating from three possible sources: a) presence of AFM1 in milk with which cheese are manufactured, as a consequence of food contaminated with AFB1 eaten by dairy cattle; b) synthesis of AFB₁, B₂, G₁, and G₂ by fungi which grow on cheese such as *Aspergillus* spp. and c) the use of dried milk with AFM1, to enrich the milk employed in cheese production. When cheese is manufactured from AFM1-contaminated milk, the toxin can be carried over into both whey and cheese (Colak, 2007).

Results showed that AFM1 was detected in 26 % in analyzed yoghurt samples with level ranging from 11.40 to 98.8 ng/kg. All positive samples of raw milk, cheeses and yoghurt are exceeding Egyptian regulations which stated that milk and dairy products should be free from AFM1, while all of them are within the US regulations (500 ng/ Kg). While 71, 75, 88 %, of positive samples in Kareish cheese, Damietta cheese and Ras cheese samples, respectively were exceeding the European Commission regulations (50 ng/Kg). Also 58 % of positive examined raw milk samples and 63% of positive examined yoghurt samples were exceeding the European Commission regulation.

Milk and other dairy products are always at risk of being contaminated with aflatoxin M1. Mycotoxin compounds are extremely stable and also dangerous in minute quantities. A few parts per billion are of concern. Once formed, they cannot be removed from the commodity. Milk, as a liquid, is a highly variable product that rapidly loses its quality and spoils if not to be treated. Since milk may be processed in numerous ways, the effects of storage and processing on stability and distribution of AFM1 are of great concern. Many researchers from different countries have carried out studies about the incidence of AFM1 in milk; many authors showed that seasonal effect influences concentration of aflatoxin M1. They reported higher concentration of AFM1 in cold seasons as compared to hot seasons, the reason being in winters mostly milking animals are fed with compound feeds and thus concentration of AFB1

increases which in turn enhances AFM1 concentration in milk (Azizollahi *et al.*, 2012).

Dairy products play a significant role in human diet since they are rich sources of bioavailable calcium and proteins. However, many of the previous studies has indicated the presence of AFM1 at high concentrations in dairy products (Tekinsen and Tekinsen 2005). The low level of AFM1 in examined yoghurt samples could 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 AFM1 with this protein causing adsorption of the toxin. Also enzymatic, microbial and particularly acid coagulation can cause degradation of AFM1 in milk (El Deeb *et al.*, 1992)

Milk and dairy products provide major nutrition's for human because many people especially children, frequently include them in their diets (Baskaya, 2006).AFM1 is resistant to thermal inactivation, pasteurization. Autoclaving and other varieties of food processing procedures (Park, 2002). It is essential to keep feeds free from contamination by AFB1 which is the mother molecule of AFM1. The concentration of AFB1 in animal feed can be reduced by good manufacturing practice and good storage practices. If preventive measure fails, AFB1 can be reduced in feed that has low concentrations or by chemical, physical or biological treatment (Signorini, 2011). It is important to reduce the occurrence of 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. 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 probably lactic acid bacteria used in yoghurt offer means of decontaminating AFM1 from milk (El-Nezami *et al.*, 1998).

CONCLUSION and RECOMMENDATION

Frequent analytical surveillance by food control agencies is highly recommended to control the incidence of mycotoxin contamination in Egypt especially in dairy products. Raw milk has to be controlled continuously for presence of AFM1 contamination. It is also extremely important to maintain low levels of AFB1 in the feeds of dairy animals. Implementing a food control system, such as the HACCP system, in the food industries is suggested as an efficient means for limiting aflatoxin contamination in the Egypt's food supply.

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مستويات الأفلاتوكسين م 1 في الألبان وبعض منتجاتها في مدينة الإسكندرية

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تعتبر الأفلاتوكسينات من السموم الفطرية عالية السمية والمسببة لكثير من الأمراض السرطانية وهذه السموم الفطرية تنتج كنواتج ثانوية أثناء التمثيل الغذائي لبعض الفطريات المعروفة من جنس الأسبرجلس (فلافس - باراسيتكس). يتحول الأفلاتوكسين ب 1 داخل جسم الحيوان إلى الأفلاتوكسين م 1 والذي بدوره ينتقل في اللبن ومن ثم تنتقل هذه السموم إلى المنتجات المصنعة من تلك الألبان. المعاملات الحرارية المختلفة سواء البسترة والتبريد والتعقيم والخبز لتلك الألبان الملوثة ليس لها تأثير يذكر على محتواها من الأفلاتوكسينات. في هذه الدراسة جمعت 150 عينة من الألبان ومنتجاتها من مدينة الإسكندرية 30 عينة من كل من اللبن الخام والجبن القريش والجبن الحمياطي والجبن الرومي والزبادي في الفترة من يناير إلى أغسطس ٢٠١٣ وذلك لبيان مدى تواجد الأفلاتوكسين م 1 وقد تبين عند الكشف على الأفلاتوكسين م 1 باستخدام الأليزا تواجده بنسب وتركيزات مختلفة فقد وجد أن 40% من عينات اللبن الخام ، 46% من عينات الجبن القريش ، 53% من عينات الجبن الحمياطي و 56% من عينات الجبن الرومي و 26% من عينات الزبادي تحتوي على الأفلاتوكسين م 1 وعند مقارنتها بالمواصفات القياسية المصرية تبين أن كل العينات الإيجابية أعلى من الحدود المسموح بها. وقد تم مناقشة الخطورة الصحية للأفلاتوكسين م 1 والمخاطر المحتملة على الصحة العامة وخاصة صحة الأطفال.