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Survey For Detection and Determination of Aflatoxins M_1 and B_1 in local Milk and Certain Dairy Products by Thin Layer Chromatographic Method.

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Abstract

90 different type of milk samples, 10 Yogurt Samples, 110 different type of cheese samples and 10 ice cream samples were collected randomly from Giza Governorate during the summer of 1998 – 1999, for detection and determination of Aflatoxins M_1& B_1 by using thin layer chromatographic method. Results revealed that the average range of Aflatoxin M_1 in milk samples amounted from 0.144 to 0.378 ng/ml. About 20 % of cows and buffaloes milk samples contained form 0.378 to 0.342 ng/ml of AFM_1, whereas about 10% of other milk samples were contaminated with 0.162, 0.288, 0.324, 0.234, 0.144 and 0.162 ng/ml for skim , Pasteurized , sterilized, UHT, powder, and baby milk, in the same order. Concentrations of AFM_1 detected in cheese samples, furthermore, varied due to the type and age of cheese being examined. 20% of cheese samples were contaminated with AFM_1 being 5.1, 3.2, 2.99, 2.099, and 2.34 ng/gm for fresh Domiati, aged Domiati, Processed and Karish cheese, respectively, whereas, 30% of the other types of cheese contained 5.88, 6.3 and 3.4 ng/gm for Roquefort, fresh Romi, and Cheddar cheese, respectively. The lowest concentration of AFM_1, of 0.116 ng/gm was detected, however, in 10% of yogurt samples. Meanwhile, 20% of ice cream samples were found to be contaminated with 2.7 ng/ml, and 10% of Feta cheese samples contained 3.3 ng/gm. It could also be appeared from results that both of cream and spread cheese were found completely free from this Aflatoxin, the lowest content of Aflatoxin detected in all of the above examined samples was 0.116, 0.162, 0.162 and 0.216 (ppb) in yogurt, skim, baby milk and cream, respectively. On the other hand, results also indicated that all milk samples were free from Aflatoxin B_1 except one sample of skim milk (out of 10) which gave positive result.

Introduction

Aflatoxin is a collective term that refers to a group of highly toxic and carcinogenic secondary metabolites produced by some common molds as *Aspergillus flavus* and *Aspergillus parasiticus* during their growth on foods and feeds or laboratory media (Marth, 1979 , Rhona *et al*., 1982 , Wood, 1989 and Piva *et al;* 1995).

Aflatoxins B_1 and M_1 are known as hepatotoxins and hepatocarcinogens and the deleterious effects in humans, especially children, of consuming AFM_1-contaminated milk are of considerable concern (Qian *et al.,* 1984, Chu, 1991). furthermore, they are potent hepatocarcinogens in several species of animals (Eaton and Callagher, 1994).

Aflatoxin M_1 is a major metabolite of AFB_1 found in milk animals that have consumed feeds contaminated with aflatoxin B_1 (Blanco *et al*., 1993; Govaris *et al*., 2001 and Ciapara *et al*., 1995).

Aflatoxin sometimes can appear in milk, cheese and other dairy products. That aflatoxin can appear in milk has been recognized since 1962 (Allcroft,R.& Carnaghan.,1962 and De Iongh *et* ***al.*** 1964). Usually some of the ingested AFB_1 is converted to AFM_1 by the liver of the cow and this form of aflatoxin is excreted in the

milk. Approximately, from 1 to 4 % of AFB_1 will be converted to AFM_1 in milk after about 12 hours after the cow consumes the toxin (Marth, 1979 and Barbieri *et al;* 1994, van Egmond and Dragacci , 2001). On the other hand, in update of survey, regulation and toxic effects of mycotoxins in Europe. Creppy (2002) observed that about 0.3 - 6.2% of Aflatoxin B_1 in animal feed is transformed to Aflatoxin M_1in milk. Aflatoxin M_1 is produced by metabolism of Aflatoxin B1. Maximum level of 0.05 and 0.5µg/kg are found in milk. The toxicity of Aflatoxin M1 is about one order of magnitude less than that of Aflatoxin B_1.

The carry-over of AFB_1 to AFM_1 is linearly correlated with milk yield and the values as 6 % have been reported at µg daily intake levels of AFB_1 (Veldman *et al.*, 1992). The carry-over of AFB_1 also to milk may vary largely from animal to animal, from day to day, and from one milking to the next (Van Egmond and Dragacci 2001), and hence the products made from such contaminated milk will also contain aflatoxin M_1. On the other hand, growth of a toxigenic *Aspergilli* on a dairy products also can result in contamination of that product with one or several of the aflatoxins that are synthesized by the mold (Marth, 1979). In countries where is necessary to import feed for animals, especially in winter, the best way to control the presence of AFM_1 in milk and milk products is to restrict the presence of the AFB_1 in the feed. The European Union has established an acceptable limit of AFB_1 in feed for animals of 10 µg/kg (Moss, 1998 and Piva *et al.*, 1989).

The concentration of AFM_1 in cow's milk is about 300 times lower than the concentration of AFB_1 consumed in the feed (WHO 1979). Shortly after the discovery of aflatoxins as feed contaminants. Allcroft and Carnaghan (1963) suggested that aflatoxin residues might occur in milk and other animal products from animals that had ingested aflatoxins in the feedstuff. De Iongh *et al.* (1964) showed by TLC on Silica gel that the toxic factor had a blue fluorescence similar to that of AFB_1, but had a much lower Rf value. A trivial name, aflatoxin M was suggested to indicate its original isolation from milk.

Material and methods

Extraction, Clean Up, Detiction, And Determination Of Aflatoxins In Different Type Of Milk And Cheese

1.*Extraction*

(According to AOAC,1995)

2. Clean up by using Column Chromatography

(According to AOAC,1995)

3. Thin layer chromatography.

(According to AOAC,1995)

a) Visual analysis For Milk.

(According to AOAC,1995)

b) Visual Analysis For Cheese:

(According to AOAC,1995)

C) Densitometric measurements:

(According to AOAC,1995)

Result and Discussions

Samples of milk and certain dairy products were randomly collected from Geza Governorate during the summer of 1998 &1999. All of these samples were subjected for analysis for the presence of Aflatoxins M_1 and B_1. Examination was carried out in duplicates using Thin Layer Chromatographic Method.

Aflatoxin M_1 (AFM_1)

It is obvious from the results presented in table (1) that the average range of Aflatoxin M_1 in milk samples amounted from 0.144 to 0.378 ng/ml. About 20 % of cows and buffaloes milk samples contained form 0.378 to 0.342 ng/ml of AFM_1, whereas about 10% of other milk samples were contaminated with 0.162, 0.288, 0.324, 0.234, 0.144 and 0.162 ng/ml for skim , Pasteurized , sterilized, UHT, powder, and baby milk, in the same order. Different types of the examined cheese samples, on the other hand, contained considerably higher concentration of AFM_1, compared with milk samples. Concentrations of AFM_1 detected in cheese samples, furthermore, varied due to the type and age of cheese being examined. 20% of

cheese samples were contaminated with AFM_1 being 5.1, 3.3, 2.99, 2.099, and 2.34 ng/gm for aged rami cheese fresh Domiati, aged Domiati, Processed and Karish cheese, respectively, whereas, 30% of the other types of cheese contained 5.88, 6.3 and 3.4 ng/gm for Roquefort, fresh Romi, and Cheddar cheese, respectively.

The lowest concentration of AFM_1, of 0.116 ng/gm was detected, however, in 10% of yogurt samples. Meanwhile, 20% of ice cream samples were found to be contaminated with 2.7 ng/ml, and 10% of Feta cheese samples contained 3.3 ng/gm. It could also be appeared from results in the same table that both of cream and spread cheese were found completely free from this Aflatoxin, the lowest content of Aflatoxin detected in all of the above examined samples was 0.116, 0.162, 0.162 and 0.216 (ppb) in yogurt, skim, baby milk and cream, respectively, on the other hand, results in table (1) also indicated that all milk samples were free from Aflatoxin B_1 except one sample of skim milk which gave positive result. Fresh Romi cheese and ice cream samples were also free from AFB_1.On the other hand, one sample of yoghurt, fresh Domiati cheese, cream cheese, processed cheese , spread cheese and Feta cheese were contaminated with 1.79, 4.11, 7.7, 9.2, 8.7, and 3.6 ppb (ng/gm), respectively. Two samples of Karish cheese were positive for the presence of AFB_1 and contained 5.1 ppb. Three samples (out of ten) of aged Romi, aged Domiati, and Cheddar cheese were found to contain 12.8, 7.185, and 13.9 ppb (ng/gm), respectively.

The above mentioned results came in agreement with Kiermeier and Mucke (1972), which made a survey of commercial raw milk samples in west Germany and found that among 36 milk samples from individual factories, during Feb. to April, only 12 samples were contaminated with aflatoxins in concentration of 0.04 – 0.25 µg/L. Aflatoxin M_1 was detected by Sabino, *et. al*,.(1989) in only one sample of commercially available cows milk, while those from the farms were found to contain a minimum of 0.1µg/L and a maximum of 1.68 µg/L. Kiermeier *et al.* (1977) also reported that 79 sample (19%) out of 419 milk, delivered to his institute dairy plants contained aflatoxin M at levels ranging from 0.02 to 0.54 µg/L. Sylos *et al.* (1996), on the other hand, examined 152 samples of pasteurised milk, powder milk, Chesse, and yoghurt, collected from Groceries and supermarkets in Brazil, during 1989-1990, and found four samples of the batch were contaminated with AFM_1 at 73 –370 µg/L.

Suarez (1988) studied the presence of AFM_1 in 47 samples of commercial UHT milk in northwest Spain and found 14 were positive for AFM_1 29 were negative and 4 (8.5%) were doubtful. Similarly, Karaioannoglou ***et al.*** (1989) found the toxin in 4 samples of raw milk (4%) at levels of 0.10-0.13 ug/kg. Markaki and Melissari, (1997) stated that thirty-two samples of pasteurised milk contained aflatoxin M_1 at levels of 2.5-5 ng/l, none contained more than 5 ng/l, while 31 contained only traces of aflatoxin (0.5-1 ng/l).

80 samples of fresh cheese, 77 of hard cheese, 65 of Camembert and 134 of process cheese were tested in West Germany by Polzhofer, (1977), who found that all types of cheese were positive for AFM_1 and hard cheese had the most AFM_1.In Kuwait, 54 samples of fresh full cream and skimmed milk, powdered milk, yoghurt, and infant formula were analysed for aflatoxin M1 (AFM1) by HPLC. 28% were contaminated with AFM1 with 6% being above the maximum permissible Limit of 0.2 ug/1. Three fresh cow milk samples collected from a private local producer showed the highest level of 0.21 ug/1. (Srivastava et al.(2001)

Nekove *et al* (1991) tested 395 samples of milk and milk products (dried milk, butter, processed cheeses, and the infant food using TLC and Spectrofluorimetry methods. 15.6% of samples were contaminated with aflatoxins. The most frequent contaminated was with AFB_1 with level ranging from 0.1–12.8 µg/kg. Less frequently a combination of AFB_1 and AFG_1 (1.8–12.7 µg/kg) was found, and 3 dried milk samples AFM_1 (0.2 – 0.4 µg/kg) was detected.

Cirlli *et al*. (1989) found that 18% of Italian cheese samples were contaminated

with AFM_1 280 – 1300 ppb, and 45 with AFM_2 340 – 870 ppb. Barrios *et al*. (1996) analysed 9 fresh cheese, 9 semi-cured or semi-ripened and 17 ripened for the presence of aflatoxin M_1 by HPLC. In 16 of 35 samples (45.71%) the presence of AFM_1 was detected in concentrations ranging between 20 and 200 ng/g of cheese. In positive cases, the mean levels of AFM_1 were 105.33 ng/g in ripened cheeses, 73.80 ng/g in simi-ripened cheeses and 42.60 ng/g in fresh cheeses.

Karaioannoglou *et al*. (1989) also noted that the toxin was not detected in any of the examined Feta or Teleme cheese samples.

A seasonal trend in milk contamination with AFM_1 was noted in a few of surveys, with lower AFM_1 level in milk in the summer months. This phenomena was attributed to the fact that the cows are receiving less concentrated feeds in the summer when they are grazing. In almost all surveys, positive samples were found with AFM_1 levels exceeding 0.05 µg/kg. In various studies, samples were reported with level in the rang of proposed tolerance values for AFM_1 in milk, with the exception of infant milk, for which lower tolerance levels have been mandated (Van Egmond 1994).

Abu-Sree (1997) claimed that only from 1 - 4 % of ingested AFB_1 would appear as AFM_1 and 2% of samples were contaminated with AFM_1 mainly in cheese from Cairo and Domiat governorates, average concentration of 2.05 µg/kg. El-Deeb (1980) observed that the concentration of AFM_1 in milk ranged between 2.08 and 5.82, and between 2.04 and 7.22 µg/kg for cow and buffalo milk, respectively.

Fremy (1982) detected (in France) between 0.05 and 0.5 µg AFM_1 in milk, with the amount varying according to season. On the other hand, Balata *et al.* (1996) detected AFM_1 in 25% of camel's milk samples, with a mean value of 0.55 µg/L (0.3-0.85). Average level of AFM_1 in milk samples was 1.159 µg/litre (range from 0.1-3.5µg/L) Rajan *et al.*(1995); Kawamura *et al*. (1994) examined the presence of AFM_1 in 58 dried milk samples using ELISA and 4(US) 21(Chinese) and 1 (Polish)samples whish gave positive results for AFM_1, with an average content of AFM_1 of 95.5,102.8 and 85.0 pg/g, respectively.

The existence of AFB_1 in milk may be due to contaminated feedstuffs not completely metabolised by cow to AFM_1, thus AFB_1 will be excreted in milk, or from milk contamination after milking by AFB_1. Aflatoxin B_1 can get into dairy products from contaminated milk and from growth of the toxigenic Aspergilli on dairy products during the storage period. So we recommended to test milk and dairy products as a routin examination in milk in Egypt.

Table (1): Survey for Detection and Determination of Aflatoxins M_1 and B_1 in Local Milk and dairy products by Thin Layer Chromatography.

Type of Milk and Dairy Products.	Number of Samples	Average of Aflatoxins Content (ppb)/L or Kg		Number of Positive Samples.	
		M₁	B_1	***M₁***	B_1
Skim Milk	10	0.162	3.54	1	1
Cream	10	0.216	3.85	2	1
Cow's Milk	10	0.378	00	2	0
Past.Milk *	10	0.288	00	1	0
Steri.Milk **	10	0.324	00	1	0
UHT Milk ***	10	0.234	00	1	0
Buffalo' s Milk	10	0.342	00	2	0
Powd. Milk	10	0.144	00	1	0
Baby Milk	10	0.162	00	1	0
Yogurt	10	0.116	1.79	1	1
Roquefort Cheese	10	5.880	12.8	3	4
(Fresh) Romi Cheese	10	6.300	00	3	0
Aged Romi Cheese	10	5.100	12.8	2	3
Fresh Domiat Cheese	10	3.300	4.11	2	1
Aged Domiati Cheese	10	2.999	7.185	2	3
Cream Cheese	10	00	7.7	0	1
Processed Cheese	10	2.099	9.2	2	1
Karish Cheese	10	2.340	5.1	2	2
Spread Cheese	10	00	8.7	0	1
Feta Cheese	10	3.300	3.6	1	1
Cheddar Cheese	10	3.400	13.9	3	3
Ice Cream	10	2.700	00	2	0

**Pasteuraized Milk*
*** Sterilized Milk*
**** Ultera High Temperature*

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عمل حصر وتقرير للأفلاتوكسين م$_1$، ب$_1$ فى الألبان الخام والألبان المصنعة بواسطة التحليل الكروماتوجرافى

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تم إجراء حصر للألبان المحلية ومنتجاتها من اسواق محافظة الجيزة (ج . ع . م) للكشف عن مدى وجود الافلاتوكسينات م 1 , ب1 بواسطة طريقة كروماتوجرافيا اتلطبقة الرقيقة (TLC)

أ- أفلاتوكسين م$_1$:-

1- جميع الألبان مجال الإختبار والدراسة كانت ملوثة بالأفلاتوكسين م$_1$ فى حدود 114و إلى 378و جزء فى البليون أى ميكروجرام / لتر، حيث كان حوالى 20% من ألبان الأبقار والجاموس ملوثة فى حدود 387و للأبقار، 342و الجاموس وحوالى 10% من معظم الأبان المختلفة كانت تحتوى على م$_1$ بنسبة 162و ، 288و، 324و، 234و، 144و، 162و، ميكروجرام/لتر لكل من اللبن الفرز واللبن المبستر واللبن المعقم واللبن المعامل بالبسترة الفوقية واللبن الجاف ولبن الأطفال على التوالى.

2- بالنسبة للأنواع المختلفة من عينات الجبن مجال الإختبار كانت تحتوى على تركيزات أعلى من الأفلاتوكسين م$_1$ بالمقارنة بعينات الألبان المختبرة كما إختلف تركيز وجودة فى عينات الجبن تبعاً لنوع الجبن وطرق ومدة تخزينة.

3- كانت 10% من عينات اليوجروت تحتوى على 116و جزء فى البليون وهى تمثل أقل تركيز من الأفلاتوكسين م$_1$ وكذلك كانت 20% من عينات الأيس كريم تحتوى على 2.7 جزء فى البليون بينما عينات الجبن الفيتا كانت تحتوى على 3.3 جزء فى البليون.

4- كلا من القشدة وجبن الإسبريد كانت خالية تماماً من تلك السموم الفطرية.

5- أقل محتوى من الأفلاتوكسين كان فى اليوجروت واللبن الفرز ولين الأطفال والقشدة بنسب 116و، 162و، 162و، 216و، جزء فى البليون على التوالى بالنسبة لجميع العينات المختبرة.

ب- أفلاتوكسين ب$_1$:-

1- أظهرت النتائج أن جميع الألبان كانت خالية من الأفلاتوكسين **ب**$_1$ ما عدا عينة واحدة فقط من10عينات من اللبن الفرز كانت ملوثة بة.
2- الجبن الرومى الطازج والأيس كريم كان أيضاً خالياً من الأفلاتوكسين **ب**$_1$ بينما عينة واحدة من بين 10عينات من اليوجورت والجبن الدمياطى الطازج وجبن القشدة والجبن المطبوخ وجبن الاسبريد وجبن الفيتا كانت ملوثة به بنسب 1.97، 4.11، 7.7، 9.2، 8.7، 3.6، جزء فى البليون على التوالى.
3- عينتان من بين عشرة عينات من جبن القريش كانت تحتوى على حوالى 5.1 جزء فى البليون منه.
4- وجد فى 3 عينات من بين 10 عينات من كلا من الجبن الدمياطى القديم، والجبن الرومى القديم وجبن تشيدر كانت ملوثة بنسب 7.581، 12.8، 13.9 جزء فى البليون على التوالى، بينما كانت 4 عينات من 10عينات جبن الريكفورت تحتوى على 12.8 جزء فى البليون (ميكروجرام/لتر).
لذا تنصح هذه الدراسة بأجراء فحص على اللبن ومنتجاته للتأكد من خلوة من منتجات الفطريات.