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 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 carcinogetic 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 AFB₁ will be converted to AFM₁ 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 B₁ in animal feed is transformed to Aflatoxin M₁ in milk. Aflatoxin M₁ 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 B₁.

The carry-over of AFB₁ to AFM₁ is linearly correlated with milk yield and the values as 6 % have been reported at µg daily intake levels of AFB₁ (Veldman et al., 1992). The carry-over of AFB₁ 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 M₁. 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 AFM₁ in milk and milk products is to restrict the presence of the AFB₁ in the feed. The European Union has established an acceptable limit of AFB₁ in feed for animals of 10 µg/kg (Moss, 1998 and Piva et al., 1989).

The concentration of AFM₁ in cow’s milk is about 300 times lower than the concentration of AFB₁ consumed in the feed (WHO 1979). Shortly after the discovery of aflatoxins as feed contaminannts, 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 AFB₁, 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, Detection, 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 M₁ and B₁. Examination was carried out in duplicates using Thin Layer Chromatographic Method.

**Aflatoxin M₁ (AFM₁)**

It is obvious from the results presented in table (1) that the average range of Aflatoxin M₁ 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 AFM₁, 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 AFM₁, compared with milk samples. Concentrations of AFM₁ 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 M$_1$ (AFM$_1$) by HPLC. 28% were contaminated with AFM$_1$ with 6% being above the maximum permissible Limit of 0.2 ug/l. Three fresh cow milk samples collected from a private local producer showed the highest level of 0.21 ug/l. (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.

Cirilli et al. (1989) found that 18% of Italian cheese samples were contaminated
Survey For Detection and Determination

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 range 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 which 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\textsubscript{1} and B\textsubscript{1} in Local Milk and dairy products by Thin Layer Chromatography.

<table>
<thead>
<tr>
<th>Type of Milk and Dairy Products.</th>
<th>Number of Samples</th>
<th>Average of Aflatoxins Content (ppb)/L or Kg</th>
<th>Number of Positive Samples.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>(M_1)</td>
<td>(B_1)</td>
</tr>
<tr>
<td>Skim Milk</td>
<td>10</td>
<td>0.162</td>
<td>3.54</td>
</tr>
<tr>
<td>Cream</td>
<td>10</td>
<td>0.216</td>
<td>3.85</td>
</tr>
<tr>
<td>Cow’s Milk</td>
<td>10</td>
<td>0.378</td>
<td>0.00</td>
</tr>
<tr>
<td>Past.Milk *</td>
<td>10</td>
<td>0.288</td>
<td>0.00</td>
</tr>
<tr>
<td>Steri.Milk **</td>
<td>10</td>
<td>0.324</td>
<td>0.00</td>
</tr>
<tr>
<td>UHT Milk ***</td>
<td>10</td>
<td>0.234</td>
<td>0.00</td>
</tr>
<tr>
<td>Buffalo’ s Milk</td>
<td>10</td>
<td>0.342</td>
<td>0.00</td>
</tr>
<tr>
<td>Powd. Milk</td>
<td>10</td>
<td>0.144</td>
<td>0.00</td>
</tr>
<tr>
<td>Baby Milk</td>
<td>10</td>
<td>0.162</td>
<td>0.00</td>
</tr>
<tr>
<td>Yogurt</td>
<td>10</td>
<td>0.116</td>
<td>1.79</td>
</tr>
<tr>
<td>Roquefort Cheese (Fresh) Romi Cheese</td>
<td>10</td>
<td>5.880</td>
<td>12.8</td>
</tr>
<tr>
<td>Aged Romi Cheese</td>
<td>10</td>
<td>6.300</td>
<td>0.00</td>
</tr>
<tr>
<td>Fresh Domiat Cheese</td>
<td>10</td>
<td>3.300</td>
<td>4.11</td>
</tr>
<tr>
<td>Aged Domiati Cheese</td>
<td>10</td>
<td>2.999</td>
<td>7.185</td>
</tr>
<tr>
<td>Cream Cheese</td>
<td>10</td>
<td>0.00</td>
<td>7.7</td>
</tr>
<tr>
<td>Processed Cheese</td>
<td>10</td>
<td>2.099</td>
<td>9.2</td>
</tr>
<tr>
<td>Karish Cheese</td>
<td>10</td>
<td>2.340</td>
<td>5.1</td>
</tr>
<tr>
<td>Spread Cheese</td>
<td>10</td>
<td>0.00</td>
<td>8.7</td>
</tr>
<tr>
<td>Feta Cheese</td>
<td>10</td>
<td>3.300</td>
<td>3.6</td>
</tr>
<tr>
<td>Cheddar Cheese</td>
<td>10</td>
<td>3.400</td>
<td>13.9</td>
</tr>
<tr>
<td>Ice Cream</td>
<td>10</td>
<td>2.700</td>
<td>0.00</td>
</tr>
</tbody>
</table>

*Pasteurized Milk
** Sterilized Milk
*** Ultra High Temperature

References


36. **Sylos, CM.de; Rodriguez, Amaya. DB; Carvatho. PRN;De. Sylos,CM** (1996), Occurrence of Aflatoxin M₁ in milk and Dairy Products Commercialized in Campinas Brazil Food-Additives and Contaminants, 13:2,169-172.


عمل حصر وتقدير للأفلاتوكسين A، ب

في الألبان الخام والألبان المصنعة بواسطة التحليل الكروماتوغرافي: طه عبد الحليم نصيب، سوزان نصف جرجس، محمود محمد مطاوع

1- أستاذ ميكروبيولوجيا الألبان كلية الزراعة جامعة المنصورة, 2- الهيئة القومية للرقابة والبحث الدوائي

تم إجراء حصر للألبان المحلية ومنتجاتها من اسواق محافظة الجيزة (ج. ع. م) للكشف عن مدى وجود الأفلاتوكسينات A، B1 بواسطة طريقة كروماتوغرافيا اللطيئة الرقيقة (TLC)

A- أفلاتوكسين A:
1- جميع الألبان مجال الاختبار والدراسة كانت ملوثة بالأفلاتوكسين A في ححدود 114و إلى 378و جزء في الليمون أي ميكروجرام / لتر، حيث كان حوالي 20% من ألبان الألبان والجاموس ملوثة في ححدود 387و للألبان، 342و للكماس وحوالي 10% من معظم الألبان المختلفة كانت تحتوي على A بنسبة 62و، 288و، 324و، 234و، 144و، 162و، ميكروجرام / لتر لكل من الليم الفرز والليم المشتر والليم المعمر والليم معمر مع اللبن معامل البسترة الفوقية والليم الجاف والليم الطفل على التوالي.
2- بالنسبة للفئات المختلفة من عينات الجبن مجال الاختبار كانت تحتوي على تركيزات أعلى من الأفلاتوكسين A، B1 بالنسبة عينات الألبان المختبرة كما اختفت تركيز وجودة في عينات الجبن تبعاً لنوع الجبن وطرق ومدة تخزينه.
3- كانت 10% من عينات البوجروت تحتوي على 116و جزء في الليمون وهي تمثل أقل تركيز من الأفلاتوكسين A، B1، وكذلك كانت 20% من عينات الليم كريم تحتوي على 2.7 جزء في الليمون بينما عينات الجبن العليا كانت تحتوي على 3.3 جزء في الليمون.
4- كلا من الفئات وجبان الإمسرد كانت خالية تماماً من تلك النمر الفضية.
5- أقل محتوى من الأفلاتوكسين كان في البوجروت والليم الفرز والليم الجاف والليم الطفل بنسبة 116و، 62و، 162و، 216و، جزء في الليمون على التوالي بالنسبة لجميع العينات المختبرة.

B- أفلاتوكسين B1:
1- أظهرت النتائج أن جميع الأثيلان كانت خالية من الأفلاتوكسين، ما عدا عينة واحدة فقط من 10 عينات من اللبن الفرز كانت ملوثة به.

2- الجين الرومي الطازج والأبيض كريم كان أيضاً خالياً من الأفلاتوكسين، بينما عينة واحدة من بين 10 عينات من البويجورت والجين الدمياطي الطازج وجبين القشدة والجين المبطبوخ وجبين الأسبريد وجبين الفيتا كانت ملوثة به بنسبة 1.97، 4.11، 7.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7، 9.2، 8.7.

3- عينتان من بين عشرة عينات من جين القريش كانت تحتوي على حوالي 5.1 جزء في الفولتن منه.

4- وجد في 3 عينات من بين 10 عينات من كلا من الجين الدمياطي القديم، والجين الرومي القديم وجبين تشيد كانت ملوثة بنسبة 7.581، 12.8، 13.9 جزء في البيليون على التوالي، بينما كانت 4 عينات من 10 عينات جين الرفيفور تحتوي على 12.8 جزء في البيليون (ميكروجرام/لتر).

لذا تتصد هذه الدراسة بأجراء فحص على اللبن ومنتجاتة للتأكد من خلوة من منتجات الفطريات.