INCIDENCE OF AFLATOXINS B_1 AND M_1 IN RAW MILK AND SOME CHEESES

Abdel-Khalek, A.

Food Hygiene and Control Dept. Faculty of Vet. Med., Mansoura Univ.

ABSTRACT

A total of 150 random samples of market raw cows milk, hard cheese, and processed cheese (50 each) were collected from different groceries and supermarkets at Mansoura city, and investigated for the presence of aflatoxins B1 and/or M1 as well as the thermostability of both toxins against laboratory pasteurization of contaminated milk samples at 63°C for 30 min.

One hundred and twenty one (80.66%) of 150 samples were contaminated with aflatoxin(s), 35 (23.33%) samples were polluted with AFB_1 alone, 33 (22.00%) samples had AFM_1 alone, and 53(35.33%) samples contained AFB_1 and AFM_1. Of all examined samples, 35(70%) milk, 45 (90%) hard cheese, and 41(82%) processed cheese samples were contaminated with aflatoxin (s). The averages of AFB1 and AFM_1 levels were 1.06 ± 0.23 and 1.16 ± 0.20µg/L of milk, 7.54 ± 1.46 and 3.88 ± 0.90 µg/kg of hard cheese, and 13.11 ± 2.70 and 14.06 ± 2.36 µg/kg of processed cheese samples, respectively. All contaminated cheese samples contained AFB_1 and/or AFM_1 by levels more than both Egyptian and FDA limits (nil and 0.5 µg/kg for each toxin), while 20 (40%) and 21 (42%) of aflatoxin- contaminated raw milk samples harboured toxin quantities higher than FDA limits for AFB_1 and AFM_1 consecutively (0.5 µg/L for each toxin).

As regards the effect of pasteurization of aflatoxin- contaminated milk samples at 63°C for 30 min., there were a negligible percentages of reductions ranged from 4.9 to 7.8% for AFB_1 and 0.6 to 3.1% for AFM_1. Public health significance of recognized aflatoxins as well as a suggested measures for minimizing or resolving such a world wide problem were discussed.

INTRODUCTION

Milk and its products are the most popular food commonly used due to their high nutritive value. Such food are liable to contaminate with different fungi from different sources during some stages of production, processing , handling and storage **(Bullerman, 1979)**. Such contamination makes them unfit for human consumption or even a dangerous source of infection among consumers and a potential health hazard caused by mycotoxins production.

Mold and yeast are widely distributed as environmental contaminants of air, dust, water and soil. The milk becomes subjected to mold contamination from these sources **(Ahmed et al., 1984; Farghaly, 1985 and Rosenzweing et al., 1986)**. Cheeses are undoubtedly exposed to further contamination besides that already in the milk ; from air , brine tank, shelves, and packaging materials **(Frazier, 1976)**. Cheese may serve as a substrate to Aspergillus flavus and other mycotoxins producing molds **(Abouzeid et al., 1996; Lopez Diaz et al., 1996 and Aman, 1998)**.

The presence of mycotoxins in milk and its products may be the result of contamination of feedstuffs consumed by dairy cattle such as aflatoxin M1, metabolite of aflatoxin B1, or direct contamination of dairy products by fungi, in particular cheese **(Van Egmond, 1989)**. The level of aflatoxin production in milk could be affected by various milk processing as pasturization, sterilization, freezing , separation, churning, concentration and drying **(Manorama and Singh, 1995)**.

AFM1 is responsible for serious public health hazards. Its highly toxic, mutagenic, teratogenic and carcinogenic compound that have been implicated as caustive agent in human hepatic and extrahepatic carcinogenic **(Massey et al., 1995 and Salwa Aly, 1999)** also, AFM1- lead to distinct kidney necrosis.

Owing to the thermostability of both aflatoxins B1 and M1 as well as hazard effects on the public health, the present study was planned for quantitative estimation of both toxins in market raw milk, hard cheese and processed cheese. The effect of heat treatment on the degradation of aflatoxins- were also studied.

MATERIALS AND METHODS

One hundred and fifty random samples of market raw milk, hard cheese and processed cheese (50 each) were collected from different supermarkets and groceries in Mansoura City. The samples were transferred to the laboratory with a minimum of delay. Each milk sample was subdivided into two subsamples the first was used for aflatoxins estimation and the second was prepared for heat treatment .

1-Estimation of aflatoxins:

Milk and cheese samples were analyzed for detection of aflatoxins B1- and M1 by the method of the Association of Official Analytical Chemists **(AOAC, 1984)** for dairy products using high performance liquid chromatography as reported by **Richard and Lyon (1986)**.

2-Effect of heat treatment on the degradation of Aflatoxins in milk samples:

100 ml of aflatoxin contaminated raw milk samples were laboratory pasteurized at 63°C for 30 min. and investigated quantitatively for both toxins AFB1 and AFM1 according to **AOAC (1984).**

RESULTS AND DISCUSSION

Inspection of data in table (1) show that the aflatoxin B1 can be recognized alone in 9 (18%) milk, 14 (28%) hard cheese , and 12 (24%) processed cheese samples, while aflatoxin M1 was detected alone in 10 (20%), 12 (24%), and 11 (22%) samples, meanwhile, both toxins were determined together in 16 (32%), 19 (38%), and 18 (36%) samples, respectively.

Many workers determined AFM1 in raw milk samples by extremely higher incidences **(Aman et al., 1993, Hassanin 1996, Galvano et al., 1998 and Kim et al. 2000)** while the others recognized the same toxin by a lower incidences **(Sabino et al., 1989 and Pilsbacher, 1995)**. On the other hand, the percentages of AFM1 contaminated milk samples were similarly obtained by **Piva et al., (1985), Margolles et al., (1990) and Saitanu (1997)** however, several researchers failed to detect the AFM1 residue in raw milk **Oliviero et al. (1987)**. These wide variations in the percentages of AFM1- contaminated milk samples are certainly confined to similar variations of the animal feeds contamination with the parent AFB1 **(Rodricks and Stoloff, 1977)**. Presence of AFB1 and/or AFM1- residues in milk and cheese samples are undoubtedly derived from the injurious AFB1 levels that usually found in animal feeds (corns, ground cottonseed cake, mouldy bread, wheat bran, and others) as a result of growth of toxigenic A. flavus and A. parasiticus strains; about 1% of feed AFB1 excreted with milk in the forms of AFB1 and AFM1 **(Rashda and Syed 1993).**

As regards the presence of AFB1 residue in raw milk, although the literatures dealt with such contamination are severely deficient, the raw milk samples tested in this work were AFB1 _ contaminated by incidence nearly similar to AFM1- polluted samples. However, aflatoxin B1 residue can be detected in milks by **Rashda and Syed (1993), Saad and Zaky (1995) and Dhand et al. (1998).**

Concerning the occurrence of AFB1 and/or AFM1 in examined cheeses, results in table (1) revealed that the percentages of either AFB1-or AFM1- contaminated cheese samples were higher than that obtained for raw milk samples, and this variance is surely attributed to that the aflatoxin B1 in cheeses does not originate only from polluted milk as a residue besides AFM1, but also from toxic mould growth as an indicator of direct cheese contamination. Furthermore, AFM1 of milk does not degrade after its conversion to cheese curd, but occurs linked with casein to increase by about 4-fold in cheese higher than the original amount of the milk toxin **(Wiseman and Marth ,1983 and Yousef and Marth , 1989)**. Although **Kivanc (1990)** failed to detect AFM1 in cheeses, many investigators could recognize it with AFB1 in different cheeses **(Truckesess and Page 1986, and Barbieri et al., 1994)** by higher incidence, while others investigators, **Abouzeid et al., (1996) and Barrios et al., (1996)** could detect the toxins by lower incidence than that obtained in this study. Reviewing the percentages of aflatoxin- contaminated cheeses reported in table (1) revealed that the hard cheese samples were the highest followed by processed cheese (Table 1). This may be attributed to the improper storage of hard cheese which favours the growth of toxic A.flavus strains and production of the toxin.

As regards the AFB1 contamination levels per liter milk or kilogram cheese, table (2) showed that this toxin was found in milk at concentration ranged from 0.19 to 1.77 with an average of 1.06±0.23μg/L and ranged from 3.95 to 8.38 with a mean value of 7.54±1.46 μg/kg hard cheese, and ranged from 6.06 to 29.07 with an average of 13.11±2.70 μg/kg in processed cheese samples. The highest AFB1 levels were detected in processed cheese samples followed by hard cheese, while in the milks were the least. The AFB1 levels ranged from 0.19 to <1 μg/L in 12% of milk samples, while 1-1.77 μg/L in 38%. The quantities of AFB1 in hard cheese ranged from 3.95 to 6 μg/kg in 14 (28%) samples and >6-8.38 μg/kg in 19 (38%) samples. Furthermore, the toxin levels in processed cheese samples lay between 6.06 and 12 μg/kg in 17 (34%) samples, >12-18 μg/kg in 8 (16%) samples >18-24 μg/kg in 2 (4%) samples , and >24-29.07 μg/kg in 3 (6%) samples (Table 2). Approximately similar AFB1 amounts in raw milk were found by **Rashda and Syed (1993) and Saad and Zaky (1995)**.

As concerns the AFB1 contamination of tested cheeses, **El-Sawi et al., (1994)** could estimate this toxin in Egyptian cottage cheese by 104 μg/kg; the concentration was extremely higher than that obtained in this work (Table, 2). Owing to the uncontrolled manufacture of processed cheese in Egypt, many domestic brands are prepared cheese in Egypt by heat- processing of other cheese trimmings that usualy contaminated heavily with mould growth and aflatoxins.

Also the capability of toxic A. flavus strains, which are frequent mould contaminants of cheeses, to grow at room temperatures and produce aflatoxins into the cheese to a depth of about 2.5 cm, render the occurrence of expected injurious AFB1 levels in some cheeses **(El-Deeb et al., 1992)**.

Results presented in table (3) declare the occurrence of AFM1 in examined samples ranged from 0.35 to1.82 μg/l in raw milk, 2.98-6.71 μg/kg in hard cheese, and 2.02-21.16 μg/kg in processed cheese samples with an averages of 1.16–0.20 μg/L, 3.88±0.90 μg/kg, and

14.06±2.36 μg/kg, respectively; 15 (30%) milk samples contained 0.35-<1 μg/L, while 11(22%) samples contaminated with 1 to 1.82 μg/L. meanwhile about 48% of hard cheese samples contaminated AFM1-showed toxin levels lay between 2.98 and 6 μg/kg, and the 14% lay between 6 to 6.71 μg/kg, Furthermore, the AFM1 quantities were estimated in processed cheese samples by levels ranged from 2.02 to 6 μg/kg in 8 (16%) samples, >6-12 μg/kg in 11 (22%) samples, >12-18 μg/kg in 8 (16%) samples and >18 μg/kg in 2(4%) samples.

Nearly similar AFM1 quantities in raw milk were determined by **Maffeo et al., (1980)** and **Aman et al., (1993)**, while higher levels were obtained by **Margolles et al., (1990)** and **Amra (1998)**, however, lower concentrations were estimated by **Hassanin (1996)**, **Galvano et al., (1998)** and Ioannou **Kakouri et al., (1999)**. These wide variations between AFM1 levels reflects the corresponding differences in the parent AFB1 concentrations in feeds of lactating cows. Also, the AFM1 quantities in tested hard and processed cheese samples were approximately 3 to 12 fold higher than that recognized in raw milk samples (Table 3), and this relation was explained by **Wiseman** and **Marth (1983)** and **Yousef** and **Marth (1989)**.

Comparing the levels of AFB_1 and AFM_1 - contaminating milk and cheese samples obtained in the present work, with Egyptian and FDA limit, table (4) show that, in relation to AFB_1-contaminated samples, all contaminated 25 (50%) milk samples and only a portion of them: 20 (40%) milk samples contained AFB_1 levels more than the Egyptian and FDA limits (nil and 0.5 μg/L), respectively, meanwhile all contaminated cheese samples; 33 (66%) hard cheese and 30 (66%) processed cheese samples harboured AFB1 by concentrations more than both aforementioned limits. Concerning AFM_1-contaminated samples, all contaminated 26 (52%) milk samples and only a portion of them; 21(42%) milk samples were polluted by AFM_1 levels more than the Egyptian and FDA limits respectively, whereas all contaminated cheese samples; 31 (62%) hard cheese and 29 (58%) processed cheese samples contained AFM_1 by levels above both limits (Table 4).

The results of this survey reflected a rather high frequency occurrence and levels of aflatoxins in Egyptian raw milk and cheeses destined for human consumption, and this may provide a profitable explanation for the high incidence of liver cancer, cirrhosis and related diseases of the liver known to occur in Africa (**Uraguchi** and **Yamazaki, 1978**). It is generally accepted that the ingestion of aflatoxin-contaminated dairy products is harmful even with quantities lower than recommended limits due to the potent effect of such toxins (AFB_1 and AFM_1) on the human health resulting from gradual accumulation in human blood and not easily degraded or excreted. Therefore the Egyptian limits should be strictly applied for precise control of human aflatoxicosis.

Data in table (5) revealed the effect of pasteurization of both AFB_1 -and AFM1- contaminated milk samples at 63°C for 30 min. on the stability of both toxins, by a reductions of 4.9-7.8% and 0.6-3.1% from the initial AFB_1 and AFM_1 concentrations, respectively. These findings agree with those recorded by **Aibara (1978)** and **Mashaly et al., (1986)**. Higher percentage of AFM_1 in contaminated milks was reduced by pasteurization (**Gelosa** and **Buzzetti, 1994**). The percentages of AFB_1 reductions in milk samples due to pasteurization were higher than that of AFM_1, this variance may attributed to the lower melting point of AFB_1 (268-269°C) than that of AFM_1 (299-300°C) (**Moreau, 1979**).

In conclusions, aflatoxins could be detected in raw milk and cheese samples as well as processing of milk by heat treatment only destroy a little portion of AFB_1 and AFM_1 in treated milk. Therefore, the best way to deal with the problem of mycotoxins in milk and dairy products is to avoid contamination of milk through prevention of contamination of feedstuffs consumed by dairy animals as well as prevention of fungal growth in the dairy products through applying strict hygienic measures during production, packing and distribution of the products.

Table 1 : Incidence of aflatoxins B_1 and M_1 in the examined samples.

Examined samples	Raw milk (N=50)	Hard cheese (N=50)	Processed cheese (N=50)	Total samples (N=150)
B_1	9 (18%)	14 (28%)	12 (24%)	35 (23.33%)
M_1	10 (20%)	12 (24%)	11 (22%)	33 (22.00%)
B_1 and M_1	16 (32%)	19 (38%)	18 (36%)	53 (35.33%)
Total	35 (70%)	45 (90%)	41 (82%)	121 (80.66%)

Table 2 : Statisical analytical results and frequency distribution of aflatoxin B_1 in market raw milk and cheese samples.

Examined samples	Tested samples (n)	Positive samples n (%)	AFB_1 contamination level, µg per liter milk or per kg cheese		Frequency distribution of samples by AFB_1 contamination level per liter milk or per kg cheese					
			Min. Max.	Mean±SEM	<1 µg	1-6 µg	>6-12 µg	>12-18 µg	>18-24 µg	>24 µg
Raw milk	50	25 (50%)	0.19-1.77	1.06±0.23	6 (12%)	19 (38%)	-	-	-	-
Hard cheese	50	33 (66%)	3.95-8.38	7.54±1.46	-	14 (28%)	19 (38%)	-	-	-
Processed cheese	50	30 (60%)	6.06-29.07	13.11±2.70	-	-	17 (34%)	8 (16%)	2 (4%)	3 (6%)

Table 3 : Statisical analytical results and frequency distribution of aflatoxin M1 in market raw milk and cheese samples.

Examined samples	Tested samples (n)	Positive samples n (%)	AFB_1 contamination level, µg per liter milk or per kg cheese		Frequency distribution of samples by AFM_1 contamination level per liter milk or per kg cheese					
			Min. Max.	Mean±SEM	<1 µg	1-6 µg	>6-12 µg	>12-18 µg	>18-24 µg	>24 µg
Raw milk	50	26 (52%)	0.35-1.82	1.16±0.20	15 (30%)	11 (22%)	-	-	-	-
Hard cheese	50	31 (62%)	2.98-6.71	3.88±0.90	-	24 (48%)	7 (14%)	-	-	-
Processed cheese	50	29 (58%)	2.02-21.16	14.06±2.36	-	8 (16%)	11 (22%)	8 (16%)	2 (4%)	3 (6%)

Table 4 : Numbers and percentages of tested samples contaminated with AFB1 and AFM1 levels higher than limits set by Egyptian and FDA regulations.

Examined samples	sample tested (n)	AFB_1 contaminated samples		AFM_1 contaminated samples	
		>Egyptian limit (zero*)	>FDA limit (0.5µg/lor kg**)	>Egyptian limit (zero*)	>FDA limit (0.5µg/lor kg**)
Raw milk	50	25 (50%)	20 (40%)	26 (52%)	21 (42%)
Hard cheese	50	33 (66%)	33 (66%)	31 (62%)	31 (62%)
Processed cheese	50	30 (60%)	30 (60%)	29 (58%)	29 (58%)

* Egyptian limit recommended by Egyptian organizatin for standardization and quality control (1990).

** FDA (Food and Drug Administration) limit reported by Wood (1992).

Table 5 : Ranges of reduction percentages of AFB1 and AFM1 residues in contaminated milk samples at 63°C for 30 min.

Aflatoxin	Reduction %
B_1	4.9-7.8
M_1	0.6-3.1

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الملخص العربى

مدى تواجد الأفلاتوكسينات B_1 و M_1 فى اللبن الخام وبعض أنواع الجبن

عـــادل عبدالخالـق ســـيد

قسم الرقابة الصحية على الأغذية - كلية الطب البيطرى - جامعة المنصورة

تم جمع مائة وخمسون عينة عشوائية من اللبن الخام والجبن الجاف والجبن المطبوخ (٥٠ عينة لكل نوع) من الأسواق ومحلات البقالة المختلفة بمدينة المنصورة، وذلك لمعرفة مدى تواجد الأفلاتوكسينات B_1 و M_1 بالإضافة لمعرفة مدى تأثير المعاملات الحرارية علي تلك السموم فى اللبن، وقد أسفرت النتائج على تواجد الأفلاتوكسينات فى اللبن الخام والجبن الجاف والجبن المطبوخ بنسب ٧٠٪، ٩٠٪، ٨٢٪ على الترتيب بينما كل متوسط الأفلاتوكسينات B_1 و M_1 بتركيزات ٠٦ر١ : ١٦ر١ ميكروجرام / لتر بالنسبة للبن الخام وبنسب ٥٤ر٧ ، ١١ر١٣، ٨٨ر٣، ٠٦ر١٤ ميكروجرام / كجم للجبن الجاف والجبن المطبوخ على الترتيب.

قد خلصت النتائج أن جميع عينات الجبن التى تحتوى على الأفلاتوكسينات تحتوى على نسبة أعلى من الحد المسموح به حسب المواصفات القياسية المصرية ومنظمة الأغذية والأدوية بينما ٢٠ (٤٠٪) و ٢١ (٤٢٪) من عينات اللبن الملوثة بالأفلاتوكسينات B_1 و M_1 على الترتيب أعلى من الحد المسموح به حسب مواصفات منظمة الأغذية والأدوية، بالإضافة إلى دراسة معدل تكسير الأفلاتوكسينات B_1 و M_1 عند درجة ٦٣° لمدة ٣٠ دقيقة وقد تناول البحث الأهمية الصحية لتلك السموم وتأثيرها على صحة الإنسان والاشتراطات الصحية لمنع تلوث اللبن والجبن بهذه الميكروبات وسمومها.