

## **DETECTION OF AFLATOXIN M1 IN LOCAL CHEESE OF MOSUL CITY, IRAQ**

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### **ABSTRACT**

A total number of 90 (45 soft white and 45 processed) local cheese samples purchased from Mosul City supermarkets from March to June 2013 were analyzed for Aflatoxins (M1), AFM1 using competitive Enzyme-Linked Immunosorbent Assay (ELISA). Results showed that 37 soft white cheese samples (82.22%) were positive to residual AFM1 with a range between 0.000-0.470 ppb and a mean of 0.133 ppb, while all the 45 processed cheese samples (100%) were contaminated with AFM1 at a range between 0.040-0.810 ppb and a mean of 0.213 ppb the difference between mean was significant ( $p < 0.05$ ) than that of soft white cheese samples. Four soft white cheese samples (8.88%) were failing to reach the desired level of the European Communities and Codex, compared with 9 samples of processed cheese (20%) failed to reach the desired level. The public health hazard of AFM1 in milk and dairy products was discussed.

### **INTRODUCTION**

Aflatoxins are toxic metabolites produced by a variety of molds such as *Aspergillus flavus*, *A. parasiticus* and *A. nomius* (1). Aflatoxins can be present in grains and other commodities associated with human food or animal feeds. Crops may be contaminated by one or more of the four following sub-types of aflatoxins: B1, B2, G1 and G2. Aflatoxin B1 (AFB1) is the most toxic (2). According to the severity of their toxicity, mutagenicity and carcinogenic effects, aflatoxins can be arranged as

follows AFB1>AFG1>AFB2>AFG2 (3). AFB1 molecule has been categorized as class I human carcinogen (4).

Human diet may be the principal way for entrance of aflatoxins into the human body (5). Milk and dairy products are fundamental components in the human diet. Aflatoxins contamination in milk and dairy products is produced in two ways. Either directly from contamination of milk and dairy products with aflatoxins producing molds (6), or indirectly when toxins pass to milk with ingestion of feeds contaminated with aflatoxin. Aflatoxin B1 is converted by hydroxylation to aflatoxin M1 (AFM1), which is subsequently secreted in the milk of lactating animals (7). Aflatoxin M1 is quite stable towards the normal milk processing methods such as pasteurization process or in yoghurt and cheese making (6).

There is a linear relationship between the amount of AFM1 in milk and AFB1 in feed consumed by animals. It has been reported that 0.3-6.2% of AFB1 in animal feed is transformed to AFM1 and excreted in milk. AFM1 could be detected in milk 12-24 hour after the AFB1 ingestion, reaching a higher level after a few days. When AFB1 intake is stopped, the AFM1 concentration in milk decreases to an undetectable level after 72 hours (8). The presence of AFM1 in milk and dairy products is considered undesirable due to their toxic and carcinogenic properties (9).

As aflatoxins pose more serious risks to public health, certain limits of aflatoxins in foods are determined (10-12). For this reason, many countries have regulations to control the levels of AFB1 in feeds and to propose maximum permissible levels of AFM1 in milk to reduce this risk (13-19).

According to USA regulations, the level of AFM1 in milk should not be higher than 0.5 ppb (15). The European Commission (EC) has approved a maximum admissible level of 0.05 and 0.25 ppb for AFM1 in milk and cheese, respectively (17).

The mycological quality of raw milk and cheese in Iraq has not been studied. Therefore, this study aimed to investigate the presence of AFM1 in locally manufactured soft white (Arab) and processed cheese that especially sold and consumed in Mosul City (Northern Iraq) by ELISA method and to compare the results with the legal regulations for AFM1 legislated in various countries.

## **MATERIALS AND METHODS**

### **Sample collection**

A total of 90 random local hand-made soft white and factory processed cheese samples (45 samples of each) was analyzed for AFM1. All of the samples were purchased from Mosul City supermarkets from March to June 2013. All samples were stored at -20°C till analysis.

### **Method for analysis**

The competitive Enzyme-Linked Immunosorbent Assay (ELISA). was used in the current study. Commercial ELISA kit was purchased from Shenzhen Lvshiyuan Biotechnology Co., Ltd, Version 2012-02. The preparation was done as described in the ELISA kits, is as follows: 2±0.05 g of homogenized cheese sample was taken into 50 ml centrifuge tube, added to it 8 ml sample extract solution (3 parts of methanol dissolved with 2 parts of deionized water) and shaken thoroughly for 30 s, the sample was then centrifuged at above 4000 r/min at 15<sup>0</sup>C for 10 min. After that 100 µl of the supernatant was taken, and 500 µl sample redissolving solution was added (offered by the kit). To test 50 µl was taken. fifty µl of the sample or standard solution were added to separate duplicate strip wells. To these solutions 50 µl of the enzyme conjugate was added/well, then 50 µl antibody working solutions/well. All solutions were mixed, covered with a membrane and incubated at 25<sup>0</sup>C for 30 min. in the dark. After incubation liquid was poured out of microwells, then washed with 250 µl/well washing buffer for 4-5 times, 15-30 s each time. After drying , 50 µl of substrate A solution and then B solution were added/each well. After gently shaking, the plate was incubated at 25<sup>0</sup>C for 15 min. in the dark for coloration. All reactions were ended by the addition of stop solution (50 µl/well). Optical density for each well was determined by the microplate reader at the dual-wavelength of 450/630 nm within 5 min.

### **Statistical analysis:**

The data were Statistically analyzed using Sigma Stat for windows Version 3.10 (2004). The differences in the mean concentrations of AFM1 between soft white cheese and processed cheese were compared using Student t-test (20). AFM1

concentrations were obtained by using the statistical software package SPSS version 16.

### RESULTS

The percentage of positive cheese samples in both soft white and processed cheese for AFM1. Of the 45 soft white cheese samples tested, 37 samples were positive ( 82.22%) with a range between 0.000-0.470 ppb and a mean of 0.133 ppb. All the 45 processed cheese samples were contaminated with residual AFM1 (100%) with a range between 0.040-0.810 ppb with a significant difference ( $p < 0.05$ ) of 0.213 ppb, compared with that of soft white cheese samples (Table and figur1).

**Table 1: Range and mean concentrations (ppb) of aflatoxin M<sub>1</sub> in soft white (Arab) cheese and processed cheese.**

Type of examined cheese samples	No. of examined cheese samples	Range	Mean ± SE
Soft white (Arab) cheese	45	0.000-0.470	0.133± 0.0162 a
Processed cheese	45	0.040-0.810	0.213 ± 0.0260 b

\*Result are expressed as mean ± standard error of mean  
 \*Different letters are significantly different at ( $p < 0.05$ )

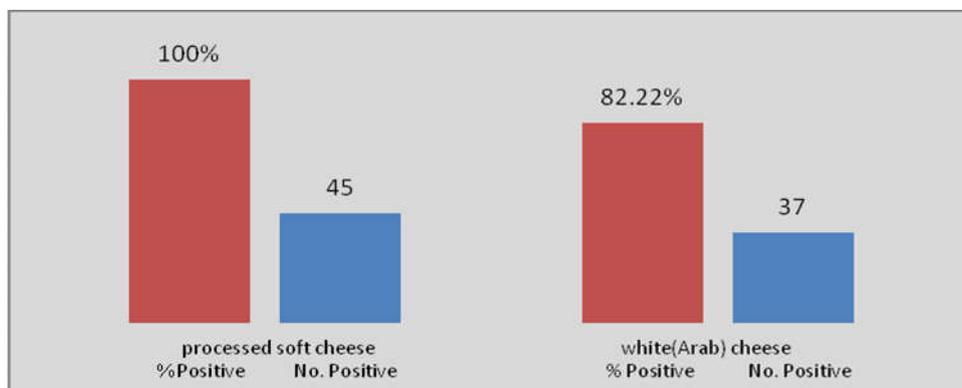


Figure 1: Number and percentage of the positive cheese samples to residual AFM1.

The occurrence of AFM1 was distributed in 37 positive soft white cheese samples ( Table 2 & 4). It is evident that 4 samples (8.88%) were failing to reach the desired level of the European Communities and Codex, defined as 0.25 ppb, [8 negative samples(17.77%) and 33 positive samples (73.33%)] were reached the desired level.

**Table 2: Occurrence of AFM1 in soft white (Arab) cheese samples in Mosul City.**

AFM1 levels ppb	Samples No.	(%)	Range
Not detected	8	17.77	0.000
< 0.050	3	6.66	0.040
0.050-0.10	7	15.55	0.051-0.100
0.101-0.250	23	51.11	0.110-0.240
> 0.250	4	8.88	0.280-0.470
Total samples	45	82.22	0.000-0.470

In case of processed cheese samples, 9 samples (20%) failed to reach the desired level of the European Communities and Codex, defined as 0.25 ppb, while 36 samples (80%) reached the desired level (Table 3 and 4).

**Table 3: Occurrence of AFM1 in processed cheese samples in Mosul City.**

AFM1 levels ppb	Samples No.	(%)	Range
Not detected	-	-	-
< 0.050	1	2.22	0.040
0.050-0.10	10	22.220	0.056-0.090
0.101-0.250	25	55.550	0.110-0.250
> 0.250	9	20.00	0.310-0.810
Total samples	45	100	0.040-0.810

**Table 4: Number and percentage of soft white (Arab) cheese and processed cheese samples exceeding the maximum permissible limits for Aflatoxin M<sub>1</sub> as proposed by many European countries (0.25 ppb).**

Type of examined cheese samples	No. of examined cheese samples	No.& percentages (%) of samples not exceeding EC maximum permissible limits	No.(%) of samples exceeding the maximum permissible limits
Soft white (Arab) cheese	45	41 (91.12%)	4 (8.88%)
Processed cheese	45	36 (80%)	9 (20%)

## DISCUSSION

Cheese as a dairy product plays a crucial role in Mosul citizens diet, since it provides a good source of calcium and proteins. Previous works conducted in Mosul City studied the presence of AFB1 and AFM1 in milk and some dairy products manufactured from the milk of cows, ewes, buffalos and goats (21-23). Other studies elsewhere revealed high concentrations of AFM1 in dairy products (10, 24, 25). Occurrence of AFM1 in cheese can originate from two possible causes. The first one, AFM1 present in raw milk because of carryovers of AFB1 from contaminated feed to milk. The second cause may be traced to the contaminated dried milk or other additives used to enrich the milk, which is being used in dairy processing plants for production of cheese (26).

In the current study it was evident that soft white (Arab) cheese( primarily hand-made from raw ewe 's milk) was contaminated with residual AFM1 at a lesser percentage and with lower mean value than the percentage of contamination and mean value of processed cheese (mainly factory produced and made from soft cheeses manufactured from milk of cows, ewes and buffalos). Raw cow's milk may be contaminated with AFM1 at higher concentrations than raw ewe's milk. This primarily belongs to that cattle depend on concentrated feeds more than sheep during winter and spring seasons (27). Furthermore, sheep have better ability for AFB1 breakdown than cattle, which finally decreases the excretion of AFM1 in their milk (28). Buffalo's milk may contain more concentrations of AFM1 than other milk types as it was often fed with cottonseed meal contaminated with aflatoxins producing molds. In addition, processed cheese also manufactured from hard and semi-hard cheese types that contain AFM1 more concentrated than in the soft cheese types (29). Curd heating and the addition of emulsifying salts during processing cheese manufacture also may increase AFM1 levels in this cheese type (30).

Theses finding are in agreement to a certain extent of the results obtained by (21) regarding the percentage of cheese contamination with AFM1 of 80% in the local soft cheese, compared to the processed ones, which contaminated with residual AFM1 at a higher level of 85%, while the mean concentrations of AFM1 reported in this study were higher than our results, as it recorded 1.13 and 2.09 ppb of AFM1 in soft white cheese and processed cheese, respectively. Several surveys performed in order to determine the AFM1 levels in milk, cheese and dairy products were in line of our

study. Of these studies, that performed by (24), in which 132 kashar cheese samples were analyzed for AFM1, 82.6% of their samples contained AFM1 between (0.050-0.690 ppb). The percentage of cheese samples, contamination with AFM1 was 81.75% in the study of (10), is in the line of soft white cheese results, when they examined 400 cheese samples (327 samples were positive), although they found a higher number of samples (110) (27.5%) exceeding the legal limits of 0.25 ppb. Similar to our percentage of soft white cheese contamination with AFM1, were those found by (25) in their study of 193 white cheese samples with a percentage of 82.4% and a range between (0.052-0.860 ppb). Closer mean of residual AFM1 in a white cheese to our study was found by (31), when they analyzed 130 cheese samples and determined the mean of 0.142 µg AFM1/kg.

Our findings were higher than those reported by (32), in which 63 cheese samples were analyzed and found that 28 (44.44%) were contaminated with AFM1 at a range of 0.007-0.202 ppb. This finding disagrees with previous study (33,35), in which all examined cheese samples were not contaminated.

According to observations, the levels of contamination of cheese by AFM1 seem to vary in many studies. These variations may be related to several reasons such as differences in cheese manufacturing procedures, geographical distribution, seasonal and hygienic management and the differences between AFM1 analyzing techniques (TLC, HPLC and ELISA) (33, 36-38).

In conclusion, according to results obtained, the incidence and contamination levels of AFM1 seem to be a serious problem for public health. For this reason, milk and dairy products should be inspected and controlled continuously for AFM1 contamination, animal feeds should be checked regularly for AFB1 and storage conditions of feeds must be taken under strict control.

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## الكشف عن ذيفان الافلا في الاجبان المحلية في مدينة الموصل ، العراق

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### الخلاصة

تضمنت الدراسة استخدام تقنية الاليزا التنافسية للتحري عن سم الافلا M1 في الجبن المحلي الأبيض الطري والمطبوخ. جمعت العينات من مناطق مختلفة من أسواق مدينة الموصل للفترة من آذار ولغاية حزيران ٢٠١٣ وبواقع (٤٥) عينة لكل نوع . أظهرت النتائج أن ٣٧ عينة (٨٢.٢٢ %) من الجبن الأبيض الطري كانت موجبة لتواجد بقايا سم الافلا M1 وبمدى تراوح بين (٠.٤٧٠-٠.٠٠٠) جزء بالبليون وبمعدل مقداره (٠.١٣٣) جزء بالبليون، في حين كانت جميع عينات الجبن المطبوخ ٤٥ عينة (١٠٠ %) ملوثة بسم الافلا M1 بمدى تراوح بين (٠.٨١٠-٠.٠٤٠) جزء بالبليون ومتوط تلوث قدره (٠.٢١٣) جزء بالبليون والذي كان أعلى معنويًا ( $p<0.05$ ) من معدل تلوث الجبن الأبيض الطري. أوضحت النتائج أن ٤ عينات من الجبن الأبيض الطري (٨.٨٨ %) تجاوزت الحد الأعلى المسموح به لسم الافلا M1 في الاجبان طبقا للمحددات الأوروبية مقارنة بالجبن المطبوخ والذي وصل فيه عدد العينات المتجاوزة ٩ عينات بنسبة (٢٠%). وقد تم مناقشة المخاطر المتعلقة بتواجد سم الافلا M1 في الحليب ومنتجاته على الصحة العامة.

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