# Aflatoxin $M_1$ Contamination in Fluid Milk Products, in Khartoum State, Sudan

**Abeer A. Fadlalla [a]; Ibtisam E.M. El Zubeir [b*] and Ayman Elnahas [c]**

[a] Department of Food Hygiene and Safety, Faculty of Environmental Public Health, University of Khartoum, Sudan. E-mail: abeerawadalamin@gmail.com

[b] Department of Dairy Production, Faculty of Animal Production, University of Khartoum, Sudan. E-mail: Ibtisamelzubeir17@gmail.com

[c] Department of Surgery and Anaethesia, Faculty of Veterinary Medicine University of Khartoum, Sudan. E-mail: ayman.elnahas@dalgroup.com

[*]**Corresponding author:** Ibtisam E.M. El Zubeir (Ibtisamelzubeir17@gmail.com)

## Abstract

In this study the aim is to investigate aflatoxin $M_1$ (AFM1) contamination levels in fluid milks offered for sale in Khartoum State, Sudan. Different milk samples were analyzed by aflasensor kits, using screening method; about 25 samples from raw and 62 samples of processed milk from 3 factories; 40 UHT, 12 flavored UHT and 10 pasteurized milk. The results showed that 85.06% of milk samples were contamination with aflatoxin $M_1$ at higher levels than those set by European Commission and Codex Alimentarius. High prevalence (88.7%) of aflatoxin $M_1$ was detected in flavored, pasteurized and UHT revealed 100%, 100% and 82%, respectively and the raw milk showed 92%. In conclusion this study suggested that the presence of high contamination of AFM1 in the samples of fluid milk products could constituted possible risk hazards for human health. Therefore, imposing regulatory limits is needed for Sudan.

*Keywords:* Aflatoxin; contamination; fluid milk.

## 1. Introduction

Milk is main food in many countries as it is good source of many nutrients, however toxic substances such as aflatoxin $M_1$ (AFM1) might also be present in it (Stoloff 1980). Aflatoxins; the naturally occurring mycotoxins; are mostly produced by either *Aspergillus flavus* (Kamkar 2005; Boudra et al. 2007) or *A. parasiticus* (Boudra et al. 2007). When ruminants eat contaminated feedstuffs with aflatoxin $B_1$ (AFB1), the AFB1 is metabolized in the rumen and then transferred into AFM1 that excreted with milk (Manetta et al. 2005; Van- eijkeren et al. 2006; Boudra et al. 2007; Prandini et al. 2008). Aflatoxins can result in toxicity and carcinogenicity, which might endanger both human and animals (Verma 2004).

Aflatoxins are detected occasionally in milk (Ghorbanian et al. 2008; Martins et al. 2007; Offiah and Adesiyun 2007). Moreover, heat-treatment, cold storage, concentrating and drying of milk will not affecting the level of AFM1 (Park 2002; Tajkarimi et al. 2008). The $M_1$ was found to contaminate many dairy products like yoghurt and cheese (Van Eijkeren et al. 2006; Atanda et al. 2007); skimmed milk (Van Eijkeren et al. 2006); UHT milk (Unusan 2006); pasteurized milk (Zinedine et al. 2007); milk powered (Ali et al. 2014) and "Wara "ice cream (Atanda et al. 2007).

According to the International Agency of Research on Cancer, the AFM1 carcinogenic is classified as category 2B; hence the contamination of milk by AFM1 is significant to public health (Boudra et al. 2007). Moreover, Sugiyama et al. (2008) reported that the Joint Expert Committee on Contamination and Food Additives in 2001 considered the carcinogenicity of AFM1 as only 1/10th compared to that of AFB1.

Many national governments and international organizations example Codex Alimentarius Commission set a legal limit for AFM1 (Food and

Agriculture Organization 2004; Sugiyama et al. 2008). Among the 60 countries who set a legal limit for $AFM_1$ in milk during 2003, EU and the United States set the two peak limits; 50 ng L$-$1 and 500 ng L$-$1, respectively (Food and Agriculture Organization 2004). In China, 500 ng L$-$1 as a legal limit for $AFM_1$ in milk has been established by government. The Egyptian authorities stated that all dairy products have to be free from $AFM_1$ (Amer and Ibrahim 2010). Moreover, the national surveys done in Brazil, United Kingdom, Iran and New Zealand to investigate the occurrence and level of contamination of raw milk with $AFM_1$ for ensuring of milk products safety (UKFSA 2001; Sassahara et al, 2005; Tajkarimi et al. 2008).

Analytical methods for aflatoxin detection include advance methods of detection including thin layer chromatography (Suzana et al. 1993; Stroke and Anklam 2000; Grosso et al. 2004) and high-performance liquid chromatography (Park, 1995). However, both methods are found laborious and time consuming for detecting aflatoxin in food (Cordova – Izquierdo et al. 2007; Pal et al. 2005). Soblev (2007) reported that commercially highly specific antibody-based tests are currently available for fast identification and measurement of aflatoxins level in food (about 10 minutes). Immunochemical methods include three types of which radioimmunoassay, enzyme-linked immunosobent assay and immunoaffinity column assay, are commonly used (Grosso et al. 2004). The affinities of either monoclonal or polyclonal antibodies for aflatoxins are the bases for validity of those tests (Salter et al. 2006). Cordova-Izquierdo et al. (2007) reported that the confirmation tequniques used for aflatoxins detection involve chemical derivatization and/ or mass spectrometry (MS). Biosensors, which are multidisciplinary tools with a huge potential for detecting and quantification of aflatoxin, prove to have positive impact on health care, bio-defense, management of food and agreconomical economy. Hence, they can be used as alternative improved detection methods (Nayak et al. 2009). Because in addition to their sensitivity; which is high; short time is needed for the detection of toxic elements (Dinçkaya et al. 2011). In the present study, Aflasensor kit (Unisensor, Belgium) as a rapid detection method was used to detect aflatoxin $M_1$ occurrence in raw milk produced by some dairy farms in Khartoum State. The prevalence of aflatoxin $M_1$ in some processed fluid milks was also examined to compare the contamination levels with the international standards.

## 2. Material and methods
### Source of milk samples
Eighty-seven milk samples were collected randomly; of which 62 processed samples were from different dairy factories (n=3) and 25 raw milk from dairy farms (n=8) located in Khartoum State, Sudan. The raw milk samples were obtained directly from bulk tank milk in sterile tubes from different farms, while the processed milk was purchased from different sales centers distributing the dairy products that supplied to them by the selected dairy factories. The objective is to determine the aflatoxin $M_1$ residues using Aflasensor kit (Unisensor, Belgium). Heatsensor was used as incubator according to heat sensor accompanied manual.

### Test procedure of aflasensor
It is rapid test for Aflatoxin $M_1$ quantification in milk that required incubation temperature of 40° C.

### Background
Aflasensor is a quantitative rapid test used for aflatoxin M1 molecules measurement in raw milk samples. The dipstick assay takes 10 minutes at 40°C without any processing, cleaning or extraction of the samples. The results can be obtained by visual observation directly with a threshold level at 100 ppt of $AFM_1$. The LOQ of the Aflasensor is 20 ppt and a quantification range of 20 to 150 ppt.

### Reaction mechanisms
Alflasensor is a competitive detection test with high affinity towards the molecules of aflatoxin $M_1$ due to its specific antibody. The two components should be used when conducting the test. A microwell with antibody that linked to gold particles in pre-determined amount is the first component. The second component is a dipstick having a set of membranes with specific capture lines. For a valid test, the upper red line (the control) should be visible after the second incubation time. The addition of the milk sample (200 µl) to the reagent from the microwell resulted in its re-suspension and hence the specific antibodies will bind the analytes; if present; during the first incubation time. By dipping the dipstick into the sample, the liquid starts running vertically on the dipstick and passes through its capture zones. The development of color at the test line indicating that the milk sample is free from $AFM_1$, However the disappearance of the coloured signal at the test capture line, indicating positive result and that the milk sample was contaminated with $AFM_1$. Based on this, intensity accuracy will determine the $AFM_1$ concentration present in the milk sample (UNISENSOR 2013).

### Aflasensor kits composition
With aflasensor milk kits, 96 measurements could be performed. It is provided with 12 pots, each containing a strip of reagent microwells and dipsticks (n=8), a micropipette (200 µl) for sampling the milk, and a positive and a standard that containing powder for reconstitution of raw milk to be tested for aflatoxins.

Additional material needed include the heat sensor and negative and positive standards reconstitution. The heat sensor was used at incubation of 40°C according to the reference supplied with the heat sensor manual. The connection of the device to a power source is via the adapter provided in the delivery package. The incubation, creation a new program and the maintenance and cleaning were performed as stated for Aflasensor (UNISENSOR 2013).

The described procedure will enable running of a single or multiple sample. However, the same conditions (incubation time and temperature) were to be used for each sample.

**The summary of the protocol**

Two hundred microliter of the milk sample was added into a reagent microwell and was mixed 10 times for homogenization. Then the sample was subjected to the first incubation time (3 minutes at 40 ˚C). The dipstick was automatically dipped into the reagent microwell and the second incubation time was continued for 7 minutes at 40 ˚C. Finally, the test lines colour intensities was read and compared with the control line for interpretation of the result.

**Visual interpretation of the result**

After checking the control line, the analysis was considered as invalid if the control line was not visible. When the top line (control) is visible, the test lines were examined by comparing the intensity of the line colour of both the control and test lines. The result was considered as negative when the test line showed darker colour compared to the control line, this means that; at the given sensitivity of the test; the milk samples is free from aflatoxin $M_1$ or the level of aflatoxin $M_1$ is lower than the stated value for the enclosed aflatoxin $M_1$ limit of detection. When the test line showed the same colour intensity or lighter colour compared to the control line, this indicates positive (+) that the contamination with Aflatoxin $M_1$ is equal or higher than 100 ppt in the examined sample. When the test line disappeared, the milk sample was considered as highly positive (++) and the concentrations of aflatoxin $M_1$ residues was more than 100 ppt as shown in Figure 1.



**Figure (1):** A valid test for Aflasensor

**Statistical analyses**

The data of the present study were illustrated on a percentage basis

## 3. Results

Table 1 illustrated that approximately 78% of milk samples were contaminated with aflatoxin $M_1$: About 55% of the processed samples from different factories and about 23% from dairy farms (raw) were positive for the presence of aflatoxin.

**Table (1): Presence of aflatoxin $M_1$ (AFM$_1$) in the milk samples**

| Type of milk | Number of samples | Positive (++) (100-150 ppt) | Positive (+) (20-100 ppt) | Negative (-) (>20 ppt) |
|---|---|---|---|---|
| Raw milk | 25 | 18 (72%) | 5 (20%) | 2 (8%) |
| Processed milk | 62 | 45 (72.6%) | 10 (16.1%) | 7 (11.3%) |
| Total | 87 | 63 (72.4%) | 15 (17.2%) | 9 (10.4%) |

(++) ≡ Strong positive (100 – 150 ppt).
(+) ≡ Positive (20-100 ppt).
(-) ≡ Negative (>20 ppt).

**Table (2): Occurrence of aflatoxin (AFM1) in fluid milk samples from different dairy factories**

| Source of milk samples | Number of samples | Positive (++) | Positive (+) | Negative (-) |
|---|---|---|---|---|
| Factory (A) | 38 | 38 (100%) | 0 (0.0%) | 0 (0.0%) |
| Factory (B) | 14 | 4 (28.6%) | 10 (71.4%) | 0 (0.0%) |
| Factory (C) | 10 | 3 (30%) | 0 (0.0%) | 7 (70%) |
| Total | 62 (100%) | 45 (72.6%) | 10 (16.1%) | 7 (11.3%) |

Table 2 showed that the fluid milk samples (both pasteurized and UHT) revealed high occurrence of aflatoxin $M_1$. The contamination was in 38 (100%), 14 (100%) and 3 (30%) of the samples collected from factory A, B and C, respectively. Moreover, Table 3 showed that aflatoxin $M_1$ was detected in 10 (100%) pasteurized milk, 12 (100%) flavored UHT milk, and 33 (82%) of plain UHT milk. Also, all samples of flavored milk samples (12) were positive for aflatoxin $M_1$ (100-150 ppt), the cacao UHT milk, strawberry UHT milk and vanilla UHT milk that were 6 (100%), 4 (100%), 2 (100%) respectively.

**Table (3): Aflatoxin M1 contamination in different types of fluid milk samples**

| Type of milk samples | Positive (++) | Positive (+) | Negative (-) | Total |
|---|---|---|---|---|
| Plain UHT milk | 23 (57.5%) | 10 (25%) | 7 (17.5) | 40 (100) |
| Flavored UHT | 12 (100%) | 0 (0.0%) | 0 (0.0%) | 12 (100%) |
| Pasteurized milk | 10 (100%) | 0 (0.0%) | 0 (0.0%) | 10 (100%) |
| Total | 45 (72.6%) | 10 (16.1%) | 7 (11.3%) | 62 (100%) |

## 4. Discussion

The presence of AFM$_1$ was detected in a concentration that ranged between 20 -150 part per billion (ppt) as shown in Table 1. The fluid milk samples were contaminated with aflatoxin $M_1$ in 55 (88.7%) of the processed milk compared to the raw milk samples (23; 92%). The obtained results revealed lower values compared to those reported by Ali et al. (2014) in Khartoum State, Sudan that showed the average concentration for AFM$_1$ was between 0.1 and 2.52 µg/kg. Also, Elzupir and

Elhussein (2010) found that 85.06% of contaminated milk samples in Khartoum State exceeded the recommended limits (0.05µg/kg and 1.5µg/kg, respectively) stated by European Communities/ Codex Alimentarius recommended limits. In Nigeria, it was also found that the average concentration for $AFM_1$ was 2.04 µg/k (Atanda et al. 2007). Similarly, in India, the contamination of $AFM_1$ was found to range from 28 to 164 µg/ l. Moreover 99% of the samples were contaminated with levels, which were found to exceed the values stated by the European Communities recommended limit (Shipra et al. 2004). In the northeast of China, Pei et al. (2009) stated that the contamination of raw milk with $AFM_1$ is serious, which need continues assessment of $AFM_1$. In Sudan, Elzupir and Elhussein (2010) showed that 95.45% of milk tested samples showed higher values for $AFM_1$ than that set by EU, the mean level of contamination was 2.07 µg/kg (0.22-6.90 µg/kg).

The present study showed that 92% of the milk samples collected from the farms were contaminated with $AFM_1$ at levels, which were higher when compared to limit set by the EU. However, the 360 raw milk samples representing 78.1% of the collected samples from China revealed contamination with $AFM_1$ with a minimum concentration of 5 and a maximum value of 123 ng L−1, which was lower than the limits set by Chinese and Codex Alimentarius. only 10% of the raw milk samples were found to exceed the European Union limit (Zheng et al. (2013). Iqbal et al. (2013) found that the occurrence of $AFM_1$ in milk samples collected from urban area (42%) was high compared with only 27% from rural farm houses in Pakistan, which exceeded the EU limit and that only 15% of the milk samples from urban compared to 8% from rural farm houses revealed higher values than the limit set by the Codex Alimentarius. On the other hand, Iqbal et al. (2011) reported significant variation between milk during the morning and evening lactation in the means of $AFM_1$ concentration (0.043 µg/ kg vs 0.028 µg/kg). They concluded that few raw samples of milk from buffalo's and cow's (16.3%) in Pakistan were above the limit set by European Union. Moreover, Kamkar et al. (2013) demonstrated that 18 (28%) of cow and 32 (52%) of buffalo raw milk samples were higher than the 50 ng/l limit. However, Rohani et al. (2011) reported that half of the milk samples examined in Iran showed higher levels of $AFM_1$ compared to the value stated by the EU, moreover the concentrations in all samples were found above the maximum allowed (0.05 µg l−1) set for liquid milk in Iran.

The $AFM_1$ concentration in 23 (92%) of the examined raw milk samples revealed a minimum value of 0.05 and a maximum level µg/kg, However the European Union stating lower value. Moreover, lower values were also reported in Iran (0.02–0.09 µg/kg, 0.000.89 µg/kg and <0.01 to 0.41 µg/L by Kamkar 2005; Gholamreza et al. 2007; Rohani et al. (2011), respectively. The level of contamination detected during the present study might be because of the contaminated feed offered for the dairy cows in Khartoum (Elzupir and Elhussein, 2010; Elteib et al. 2012; Ali et al. 2014). In order to the contamination levels of $AFM_1$ in dairy products, a limit of 15 µg/kg for aflatoxin B1 in supplementary feedstuffs for lactating dairy cattle have been established by the Commission of the European Communities (EC 2006).

The fluid milk (both pasteurized and UHT samples) showed occurrence of aflatoxin M1 in 38 (100%), 14 (100%) and 3 (30%) in the samples collected from factory A, B and C, respectively (Table 2). Similarly, Santini et al. (2013) suggested continuous monitoring for aflatoxin detection in order to reduce consumer exposure since they reported contamination of $AFM_1$ in 42% of UHT milk, cheese and milk cream samples, Moreover 83% of the $AFM_1$ positive samples showed values, which were less than 5.0 ng L−1, while the rest (17%) showed a range of 10.0 and 20.0 ng L−1.

Aflatoxin M1 was detected in 12 (100%), 10 (100%) and 33(82%) in flavored, pasteurized and UHT milk samples, respectively (Table 4) All samples were contaminated with aflatoxin (cacao, strawberry and vanilla).

Table (4): Aflatoxin $M_1$ detection in flavored UHT milk samples

| Type of milk | Positive (++) | Positive (+) | Negative sample | Total |
|---|---|---|---|---|
| Cacao UHT milk | 6 (100%) | 0 (0.0%) | 0 (0.0%) | 6 (100%) |
| Vanilla UHT milk | 2 (100%) | 0 (0.0%) | 0 (0.0%) | 2 (100%) |
| Strawberry UHT milk | 4 (100%) | 0 (0.0%) | 0 (0.0%) | 4 (100%) |
| Total | 12 (100%) | 0 (0.0%) | 0 (0.0%) | 12 (100%) |

Overall, the AFM1 ranged from concentrations of 20 to 150 ppt. These results are approaching the values with the findings in Central Anatolia, Turkey showing that 129 samples of UHT milk have mean level of 108.2 ng L−1 for $AFM_1$ with prevalence rate of 58% for the positive samples. Also 62.3% of the UHT milk commercialized in central Iran was found positive, with a mean for AFM1 (Fallah 2010). This might be because most of UHT milk prepared from powder milk in some factories in Khartoum State. Ali et al. (2014) found high occurrence of aflatoxins in milk powdered in Khartoum State in a previous study. Also, Park (2002) reported no change in the amount of AFM1 after pasteurization or sterilization of the milk. Moreover, JECFA (2001) stated that when frozen contaminated milk and its products are stored for a few months, no effect on their content of $AFM_1$ was noticed. Also, Suriyasathaporn and Nakprasert (2012) found significant variation for $AFM_1$ levels in different seasons in only one trademark brand from five commercial trademarks of pasteurized milk samples produced in different areas in Thailand. They concluded that the main cause of $AFM_1$ contamination in dairy products are the factors related to the farm management compared to environmental factors.

About 55 (88.7%) samples of the processed milk showed contamination with AFM$_1$ and the values were higher compared to that stated by EU commission (Table 1). In Sudan, most of dairy products (yoghurt, pasteurized milk and UHT milk were processed using imported milk powder. This supported Ali et al. (2014) who reported high occurrence of AFM$_1$ in milk powdered in Khartoum, Sudan. The complete removal of water during the processing of milk powder results in concentration of milk solids as well as other contaminants including the aflatoxins (JECFA 2001).

## 5. Conclusion

According to the results obtained in this study, high occurrences and concentration of contamination with AFM$_1$ in samples of fluid dairy products were found using Aflasensor, which is highly specific test for the detection of aflatoxin. This higher prevalence of aflatoxins M$_1$ in the raw or processed fluid milks might cause negative impact for public health of infants and children who consumed more milk compared to other people. Hence continuous monitoring and assessment programs should be conducted in the country, in addition regulatory limits for aflatoxin M$_1$ occurrence has to be established for all dairy products. Also, trials for control and reduction of aflatoxin contamination of the raw material and dairy ration of dairy cattle should be worked out in addition to organizing educational and awareness programs among all actors in the dairy sector on the risks and the hazards of aflatoxins is highly needed. Also, strict legislations should be implemented for the imported milk products in order to control the occurrence of AFM$_1$ and for ensuring that good quality dairy products are consumed in the country.

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