

*Full Length Research Paper*

# Prevalence of aflatoxin M<sub>1</sub> in pasteurized and ultra-high temperature (UHT) milk marketed in Dar es Salaam, Tanzania

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The aim of this study was to determine the level of aflatoxin M<sub>1</sub> (AFM<sub>1</sub>) in pasteurized and UHT milk marketed in Dar es Salaam, Tanzania. AFM<sub>1</sub> in pasteurized milk samples (75) and ultra-high temperature (UHT) milk (43) was determined by using immuno-affinity high performance liquid chromatography. AFM<sub>1</sub> was detected in 97% (115/118) of samples that consisted of 96% (72/75) of pasteurized milk samples and 100% (43/43) of UHT milk samples. About 82% of the contaminated pasteurized and UHT had AFM<sub>1</sub> levels above EU acceptable levels (0.05 µg/L). However, none of the contaminated pasteurized and UHT milk sample had levels of AFM<sub>1</sub> above the maximum recommended Codex limits (0.5 µg/L). The results indicate that the contamination of the samples with AFM<sub>1</sub> at such level could pose a serious public health problem. Thus, regular monitoring of AFM<sub>1</sub> levels in milk is important in order to protect consumers.

**Key words:** Aflatoxin M<sub>1</sub>, ultra-high temperature (UHT), milk, pasteurized milk, food safety.

## INTRODUCTION

Tanzania has the third largest livestock population in Africa comprising 25 million cattle out of which 98% are indigenous breeds (FAO, 2020). The dairy production in Tanzania is categorized into two systems: traditional system and dairy system (Munyaneza et al., 2019). Traditional system is the most dominant and it is based on both milk and meat products; dairy system is based mainly on milk production (URT, 2017). In the year 2018

about 934,628 tonnes of raw and heat-treated milk were produced in Tanzania. Milk production contributes to income, food security, nutrition and household livelihood (FAOSTAT, 2020). The sector contributes to 7.4% of total national GDP and the annual growth rate (2.2%) of the sector is considered low (FAO, 2020). Raw milk is a valuable nutritious food, highly perishable, with short shelf-life and it is an excellent medium for the growth of

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microorganisms, particularly harmful bacterial pathogens that can cause spoilage and diseases to consumers (FAO, 2021). Heat treatment of milk such as pasteurization and ultra-high temperature (UHT) allows the preservation of milk and helps to reduce food-borne illness (Melini et al., 2017).

According to FAO/WHO (1982), pasteurization is defined as a heat treatment process applied to a product such as milk with the objective of minimizing numbers of harmful micro-organisms to a level at which they do not constitute a significant health hazard with minimal chemical, physical and organoleptic changes in the product. It also extends the storage time for some products by reducing the number of spoilage micro-organisms in the product (FAO/WHO, 1982). Codex Alimentarius (2004), defined UHT treatment of milk and liquid milk products as the application of heat to a continuously flowing product using such high temperatures for such time that renders the product commercially sterile at the time of processing. When UHT treatment is combined with aseptic packaging, it results in a commercially sterile product at the heating range of 135 to 150°C for 1 s up to 4 s (Melini et al., 2017). AFM<sub>1</sub> is a heat stable compound that can survive heat treatment such as pasteurization, UHT technique and autoclaving but also AFM<sub>1</sub> may be reduced but not completely destroyed by heat treatments (Mahmoodi et al., 2019; Tahira et al., 2019).

Aflatoxins are amongst the most poisonous mycotoxins and are produced by *Aspergillus flavus*, *Aspergillus parasiticus* and *Aspergillus nomius* fungi found in soil and that can grow in plant, human food products and feeds (WHO, 2018). The most important aflatoxins in order of toxicity are B<sub>1</sub>, B<sub>2</sub>, G<sub>1</sub>, and G<sub>2</sub> (Ismail et al., 2018; Tahira et al., 2019). Aflatoxins may also be found in the milk of animals that are fed contaminated feed, in the form of aflatoxin M<sub>1</sub>, a hydroxylated metabolite of aflatoxin B<sub>1</sub>, within 12 h of contaminated feed consumption (Langat et al., 2016). Once lactating cow consume contaminated feeds with aflatoxin B<sub>1</sub> it is absorbed into the gastrointestinal tract and biotransformation occurs in the liver by cytochrome P<sub>450</sub> enzymes to form a 4-hydroxy metabolite known as aflatoxin M<sub>1</sub>, a compound soluble in water and therefore it is easily excreted in milk during milking (Daou et al., 2020; Tahira et al., 2019).

Aflatoxin M<sub>1</sub> is a hepato-carcinogen, classified as a group 1 carcinogen by International Agency for Research on Cancer (IARC Monograph, 2018). Aflatoxin M<sub>1</sub> is heat-stable and can survive pasteurization, autoclaving and thermal inactivation (Zakaria et al., 2019). The contamination of milk and milk products by aflatoxin M<sub>1</sub> has been reported in various countries such as Morocco (Mannani et al., 2021), Iran (Mahmoodi et al., 2019), Lebanon (Daou et al., 2020), Turkey (Eker et al., 2019), Pakistan (Tahira et al., 2019), and Kenya (Langat et al.,

(2016).

The occurrence of aflatoxin M<sub>1</sub> in milk in Tanzania reported earlier indicated that 92% of raw cow milk retailed in Dar es Salaam city was contaminated with aflatoxin M<sub>1</sub> (Urio et al., 2006) and 83.8% of raw cow milk from households in Singida was contaminated with aflatoxin M<sub>1</sub> (Mohammed et al., 2016). However, there is no information on aflatoxin M<sub>1</sub> contamination of pasteurized and ultra-pasteurized (UHT) milk in Tanzania, as well as the awareness of contamination. Thus, the aim of this study was to carry out surveillance of the level of contamination of aflatoxin M<sub>1</sub> in pasteurized and ultra-pasteurized marketed milk in Dar-es-Salaam, commercial capital of Tanzania. The results of this study will provide information on level of milk contamination by aflatoxins and contribute to raise awareness and efforts of food control authorities in developing strategies to ensure public safety.

## MATERIALS AND METHODS

### Sample collection

A total of 118 milk samples both ultra-high temperature (UHT) and pasteurized were purchased randomly from different mini markets and supermarkets located in Kinondoni, Ilala, Temeke, Ubungo and Kigamboni districts of Dar es Salaam region a commercial city of Tanzania during December 2020 and January 2021. This region was selected on purpose since it is the largest urban consumer market with availability of milk brands from different regions in Dar es Salaam shops/outlets. Seventy-five samples of pasteurized milk and 43 UHT milk samples were collected. The larger number of pasteurized milks collected is due to the fact that most dairies produce pasteurized milk.

The collected samples originate from two climatic zones of Tanzania, hot humid coastal zone (Tanga, Morogoro, Dar es Salaam and Zanzibar) and temperate highland zone (Kilimanjaro and Iringa). All samples were randomly purchased, coded and transported in an ice box together with their original packaging prior to laboratory analysis at the Tanzania Bureau of Standards (TBS) food laboratory in Dar es Salaam.

### Aflatoxin M<sub>1</sub> analysis

#### Reagents standards, chemicals, columns and other materials

HPLC grade acetonitrile, methanol and glacial acetic acid were obtained from Fisher Scientific UK. A standard aflatoxin M<sub>1</sub> (0.504 µg/L) solution was obtained from Biopure, Romer Labs Diagnostics GmbH, Tullin Austria. Distilled water was produced with a Milli-Q Integral 15 water purification system, France. Whatman Filter paper No. 4 (Whatman Inc., Clifton, NJ, USA) and AflaStar™ M1 R, Immunoaffinity Columns (IAC) for aflatoxin M<sub>1</sub> were from Romer Labs, Austria.

#### HPLC conditions

The HPLC system (Infinity II, Agilent technologies) with vial

sampler, Quant pump, MCT and FLD Spectra (model 1260) with excitation set at 365 nm and emission 450 nm was used. Instrument settings were: injection volume 50  $\mu\text{L}$ , pump flow rate 0.8 mL/min, run time was 6 min, HPLC analytical column 4.6 $\times$ 150 mm (Waters® Spherisorb® 5  $\mu\text{m}$  ODS1, Ireland) column oven set at 25°C, mobile phase comprised 2% acetic acid: acetonitrile: methanol (40:35:25) that was prior degassed for 20 min and run isocratically. Data acquisition and processing were done with OpenLab software (Version 3.4, Agilent technologies). Aflatoxin M<sub>1</sub> peak in the chromatogram was identified by comparing its retention time with that of the analyzed aflatoxin M<sub>1</sub> standard under the same conditions.

### Standards preparation

Exactly 1985  $\mu\text{L}$  of aflatoxin M<sub>1</sub> standard was added in 9015  $\mu\text{L}$  of aflatoxin M<sub>1</sub> mobile phase to get a stock solution of 100  $\mu\text{g/L}$ . This solution was used to prepare 5, 8 and 10  $\mu\text{g/L}$  standards by dilutions with mobile phase. The 10  $\mu\text{g/L}$  standard was further diluted with mobile phase to prepare 0.1, 0.5, 1 and 3  $\mu\text{g/L}$  standard. These seven standards were used for validation and quality control of the method.

### Extraction and quantification of aflatoxin M<sub>1</sub> by HPLC

The method for extraction, detection and quantification of aflatoxin M<sub>1</sub> in the milk samples was done in a dark room according to Behfar et al. (2012) with minor modifications. Fifty milliliters of the milk samples were measured in Teflon tubes, warmed up in the water bath (ThermoHaake IP30, Germany) set at 37°C. Samples were centrifuged at 4000 rpm for 15 min (Eppendorf 5810R, German) and the fat layer was removed completely and milk was filtered through filter paper (Whatman No. 4, UK). 10 mL of the filtered milk sample was passed through aflatoxin M<sub>1</sub> immuno-affinity column (AflaStar™ M1 R IAC column, Romer Labs, Austria) at steady flow rate of 2 to 3 mL/min. The column was washed with 10 mL distilled water (Milli-Q Integral 15 water purification system, France) two times. The column was dried by means of the syringe plunger and the toxins were eluted by 1 mL acetonitrile in two portions of 500  $\mu\text{L}$  into test tubes. The extract was evaporated with nitrogen concentrator at 50°C to dryness gently with stream of nitrogen. The residues were reconstituted with 500  $\mu\text{L}$  of mobile phase and vortexed ready for injection into HPLC system.

### Method validation

#### Quality control

Linearity of the method was determined by running a seven-point calibration curve that was prepared from standard solutions having concentrations of 0.1, 0.5, 1, 3, 5, 8 and 10  $\mu\text{g/L}$  each ten times. Peak area was plotted against concentration to give a regression equation which was used to determine aflatoxin M<sub>1</sub> concentrations. The calibration curve is described with the equation  $y = 0.4796x + 0.0044$  ( $R^2 = 0.9992$ ).

#### Recovery of aflatoxin M<sub>1</sub>

The accuracy of the method was established based on the percentage recovery, and contaminated milk which was below the

limit of detection was treated as blank sample and spiked with 1.0  $\mu\text{g/L}$  aflatoxin M<sub>1</sub> standard solution, it was then run-in triplicate parallel with the samples. Recovery was calculated as:

$$\% \text{Recovery} = \frac{\text{Observed concentration} \left[ \frac{\text{ng}}{\text{mL}} \right]}{\text{Expected concentration} \left[ \frac{\text{ng}}{\text{mL}} \right]} * 100\%$$

Recovery in spiked sample was greater than 89% (89.8, 89.4 and 90.2%) with the average being 89.8% indicating the suitability and good performance of the HPLC.

### Determination of the limit of detection and limit of quantitation of the HPLC method

The LOD and LOQ were established by analyzing successive lowest dilutions (0.1  $\mu\text{g/L}$ ) of the standard solution in the matrix. These LOD and LOQ values were related to the signal to noise ratio considering concentration that generated at 3 and 10 times, respectively of the lowest calibration point. The limits of detection (LOD) and quantification (LOQ) were 0.01 and 0.031  $\mu\text{g/L}$ , respectively. Precision of the method was determined by running the lowest standard of 0.1 ng/mL ten times for three days and precision was determined by calculating their relative standard deviation. The measurement uncertainty, expressed as relative standard deviation (RSD) was 1.35%.

### Statistical analysis

Data analysis was done with R Software (version 4.0.3, 2020), as shown in the equation:

$$Y_{ij} = \mu + \beta_i + \tau_j + \epsilon_{ij}$$

where  $Y_{ij}$  is the response (aflatoxin concentration) corresponding to the  $j$ th treatment (processing technique) in the  $i$ th zones,  $\mu$  is the overall mean,  $\tau_j$  is the  $j$ th treatment effect, and  $\beta_i$  is the  $i$ th zones effect.

Skilling-Mack's test (Chatfield and Mander, 2009) by using 'Skilling.Mack' package in R was used for testing the significance variation interaction between process (UHT and pasteurized). Kruskal-Wallis test was used for testing effect of each treatment (sample type) while its pairwise comparisons was done by using Wilcoxon rank sum test with continuity correction. The  $p < 0.05$  was considered significant. All data were summarized as mean and expressed in tables  $\pm$ SE of the mean.

## RESULTS AND DISCUSSION

### Aflatoxin M<sub>1</sub> contamination in pasteurized and UHT milk

Among the 118 UHT and pasteurized milk samples analyzed in this study, 97.5% (115/118) samples were contaminated with AFM<sub>1</sub>. This is similar to the study reported by Daou et al., (2020) in Lebanon that indicated 90.9% aflatoxin M<sub>1</sub> contamination in UHT and pasteurized milk. The results of the present study were higher than the study conducted by Nejad et al. (2019) in

**Table 1.** Aflatoxin M<sub>1</sub> contamination in pasteurized and UHT milk marketed in Dar es Salaam.

Milk type	Sample(N)	Contaminated, sample n(%)	Mean±SEM (µg/L)	Range (µg/L)
UHT	43	43 (100)	0.07±0.008 <sup>b</sup>	<LOD-0.454
Pasteurized	75	72 (96)	0.144±0.015 <sup>a</sup>	0.01-0.1

N is the total number of samples analyzed for each type of milk. n is the total number of contaminated samples for each type of milk. Mean with different superscripts are significant different at p<0.05.

**Table 2.** Incidence of aflatoxin M<sub>1</sub> contamination in pasteurized and UHT milk exceeding EU and Codex regulatory limits

Milk type	Sample (N)	Contaminated Sample (n)%	Exceed EU limits [n (%)]	Exceed codex limits [n (%)]	Range (µg/L)
Pasteurized	75	72 (96)	67 (93)	0 (0)	0.05-0.454
UHT	43	43 (100)	27 (63)	0 (0)	0.05-0.115
Total	118	115 (97)	94 (81.7)	-	0.05-0.454

Contaminated samples are all analyzed samples with value > limit of detection (LOD). N is the total number of analyzed samples for each type of milk. n is the total number of contaminated samples for each type of milk.

Hamadan province of Iran who reported that 86.3% of pasteurized and UHT milk were contaminated with aflatoxin M<sub>1</sub>. However, this study was contrary to results of the study conducted in Casablanca, El Jadida, Fez and Meknès cities in Morocco, which reported that 9 (13.4%) of pasteurized and UHT milk samples were contaminated with aflatoxin M<sub>1</sub> (Mannani et al., 2021). The discrepancy in AFM<sub>1</sub> levels might be due to differences in climatic conditions, hygiene, and precautions to prevent AFM<sub>1</sub> contamination of lactating cow feedstuff and dairy processing. The overall prevalence of aflatoxin M<sub>1</sub> contamination obtained in the present study was high which indicates the risk of chronic exposure to consumers. The high AFM<sub>1</sub> concentrations might be due to poor storage of animal feeds and poor feeding practices observed, which resulted into aflatoxin B<sub>1</sub> contamination in feeds and eventually metabolized into aflatoxin M<sub>1</sub> in milk. A study carried out by Mohammed et al. (2016) in Singida region, Tanzania reported that aflatoxin M<sub>1</sub> was detected in raw milk from household cows fed with contaminated aflatoxin B<sub>1</sub> sunflower seedcakes.

Furthermore, this study (Table 1) showed that 96% (72/75) pasteurized milk samples analyzed, were found to be contaminated with AFM<sub>1</sub>. A similar observation was made in a study conducted in Beijing and Shanghai in China where 96.2% pasteurized milk samples were contaminated with AFM<sub>1</sub> (Zheng et al., 2013). In the current study, all 100% (43/43) of UHT milk samples were contaminated with AFM<sub>1</sub>. This was similar to the study conducted in Pakistan whereby all UHT milk samples 105 (100%) were contaminated by aflatoxin M<sub>1</sub> (Tahira et al., 2019). These results also confirmed the

heat stable nature of aflatoxin M<sub>1</sub>.

The highest mean for AFM<sub>1</sub> was in pasteurized milk with a significant difference between the means at p<0.05. The obtained mean value of AFM<sub>1</sub> contamination in pasteurized and UHT milk samples was 0.144±0.015 and 0.07±0.008 µg/L, respectively, while concentration range of pasteurized and UHT milk was <LOD - 0.454 and 0.01-0.1 µg/L, respectively, shown in Table 1. This was similar to the studies reported by Lindahl et al. (2018) in Nairobi, Kenya and Xiong et al. (2018) in Henan, Hubei and Hunan provinces in China whose results indicated low mean concentration of AFM<sub>1</sub> in UHT milk and high mean concentration of AFM<sub>1</sub> in pasteurized milk. These observations might be due to the fact that, UHT milk is subjected to high temperature (above 135°C) treatments to kill harmful microbes and to increase the shelf life of milk. The UHT heat treatment may reduce AFM<sub>1</sub> concentration. This is supported by a study conducted by Omeiza et al. (2018) in Nigeria reported that high temperature treatments reduce AFM<sub>1</sub> up to 58.8% but could not be removed completely.

Ninety three percent (93%) of AFM<sub>1</sub> contaminated pasteurized milk sample in this study were found to exceed the EU regulatory limits (0.05 µg/L) and 63% of UHT contaminated milk sample were found to exceed the EU regulatory limits (0.05 µg/L) (Table 2). However, none of the contaminated samples of pasteurized and UHT milk were above the maximum Codex limit (0.5 µg/L) for AFM<sub>1</sub>.

The results obtained in this study indicated that mean values for aflatoxin M<sub>1</sub> contamination for pasteurized and UHT milk samples from hot humid coastal zone (Dar es Salaam, Tanga, Zanzibar and Morogoro) and temperate

**Table 3.** Mean concentration of contaminated UHT and pasteurized milk samples marketed in Dar es salaam from various climatic zones.

Climatic zone	Milk type	Sample (N)	Mean±SEM (µg/L)
Hot humid coastal	Pasteurized	57	0.15±0.019 <sup>a</sup>
Hot humid coastal	UHT	30	0.08±0.011 <sup>b</sup>
Temperate highland	Pasteurized	18	0.11±0.009 <sup>ab</sup>
Temperate highland	UHT	13	0.05±0.005 <sup>b</sup>

Means across the column with different statistical letters indicates statistical different at 5% significant level according to Wilcoxon rank sum test with continuity correction. N is the total number of samples analyzed for each zone.

highland zone (Kilimanjaro and Iringa) ranged from 0.05±0.005 to 0.15±0.019 µg/L. The highest aflatoxin M<sub>1</sub> mean value was in the hot humid coastal zone, while temperate highland zone had the lowest contaminated sample. In all samples from climatic zones, pasteurized milk samples had statistically higher mean values ( $p < 0.05$ ) of aflatoxin M<sub>1</sub> than UHT milk samples (Table 3). Higher AFM<sub>1</sub> concentration from hot humid coastal zones might be due to the fact that hot humid zones are characterized by high temperature and humidity which are favorable environmental conditions for fungal growth in animal feeds and production of aflatoxin B<sub>1</sub> which in turn are responsible for high levels of AFM<sub>1</sub> in milk. This is supported by the study done by Khaneghahi et al. (2019) from Iran who reported that milk samples obtained from hot humid climate areas were significantly higher in AFM<sub>1</sub> content. Hot humid climates are more favorable for the growth of aflatoxigenic fungi (*A. flavus* and *A. parasiticus*) and aflatoxin production than temperate climate (Benkerroum, 2020).

## Conclusion

In the current study, high AFM<sub>1</sub> levels were found in both UHT and pasteurized milk samples collected from supermarkets and dairy shops in Dar es Salaam city. Aflatoxicosis is still one of the main public health concerns in Tanzania that lead to health hazard in all population particularly children. There is need to reduce AFM<sub>1</sub> transmission in milk by controlling aflatoxin B<sub>1</sub> contamination in animal feed and feed ingredients by adopting Good Agriculture Practices (GAP) at farm level as well as improved storage conditions. It is important that farmers and other stakeholders of the dairy industry be educated on the potential harmful effects of AFM<sub>1</sub> on human health.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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