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Research Article

Aflatoxin M_1 Contamination of Ghanaian Traditional Soft Cottage Cheese (*Wagashie*) and Health Risks Associated with Its Consumption

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Wagashie is an unripened traditional cheese consumed in West Africa including Ghana. Being a milk product, it is unfortunately susceptible to aflatoxin M_1 (AFM_1) contamination, which is indeed a grave health challenge globally. This study evaluated AFM_1 levels and health risk characterization associated with *wagashie* ($n = 182$) sampled from different locations in Ghana. AFM_1 was measured with high-performance liquid chromatography with a fluorescence detector (HPLC-FLD). Risk assessments were also conducted using models prescribed by the Joint FAO/WHO Expert Committee on Food Additives (JECFA). Out of the 182 samples analyzed for AFM_1, 93/182 (51.1%) tested positive between the range 0.00 ± 0.00–3.60 ± 0.99 μg/kg. Risk assessments of AFM_1 using deterministic models produced outcomes that ranged between 0.11 and 3.60 ng/kg bw/day, 0.09–1.54, 0–0.0323 ng aflatoxins/kg bw/day, and 3.5×10^{-3} –0.06 cases/100,000 person/yr for estimated daily intake (EDI), margin of exposure (MOE), average potency, and cancer risks, respectively, for the age categories investigated. It was established that the consumption of *wagashie* posed adverse health effects on all age categories in the selected regions of the study because all calculated MOE values were less than 100,000. Therefore, contamination of *wagashie* with AFM_1 should be a serious public health concern and as such considered a high precedence for Ghana's risk management actions.

1. Introduction

Cheese is a milk product with high nutritional and medicinal attributes. *Wagashie* is a soft unripened cheese consumed in several parts of West Africa, mainly Nigeria (*wara* or *warankashi*), Benin (*woagachie*), and Ghana (*wagashie*) [1]. Nomadic Fulani who are pastoralists and are mainly involved in the rearing of cattle from one place to the other are involved in milk production in Ghana. *Wagashie* is a processed product obtained when there is excess raw milk as a way of preserving the excess milk for the short term mainly by the Fulani women. *Wagashie* is mainly consumed at home or widely sold on the market in some regions of Ghana where cattle are predominantly reared. It is either eaten as a whole meal or consumed in combination with some other foods.

Considered a milk product and, therefore, not an exception, *wagashie* is susceptible to AFM_1 contamination. AFM_1 is the main hydroxylated metabolite of aflatoxin B_1 (AFB_1) in the liver due to cytochrome enzymes and is detected in milk and dairy products (MDPs) [2]. Mycotoxin contamination in dairy cow nutrition is mainly caused by corn and silage [3]. Studies indicate that when a dairy cow consumes approximately 40 μg/kg of AFB_1 daily, produced as a result of feed contamination by fungi belonging to the genus *Aspergillus* (*flavus* and *parasiticus* species) [4, 5], the milk it produces contains 0.05 μg/kg (highest level) of AFM_1 (Joint FAO/WHO Expert Committee on Food Additives)

[6]. Varying according to the AFB_1 contamination level in feeds, around 0.3 to 6.2% of AFB_1 consumed by dairy animals is chemically modified into AFM_1 [7].

Remarkably, neither storage nor processing can entirely eliminate AFM_1 from milk and its products. AFM_1 has been detected in pasteurized milk, UHT milk, milk powder, milk formula, yogurt, feta cheese, white cheese, and traditional cheese, ice cream, and butter [8]. Additionally, cheese is a principal source of aflatoxins among milk products owing to the correlation of AFM_1 with the casein fraction in milk, which is practically concentrated in cheese [9, 10]. Researchers have demonstrated that the concentration of AFM_1 is about threefold greater in various soft cheeses and around fivefold greater in hard cheeses than in milk from which the cheese is manufactured [10, 11]. Kitya et al. [12] underscored that AFM_1 contamination in cheese may be exacerbated by poor processing methods of milk.

Aflatoxins have been reported to concomitantly work to worsen the risk of hepatocellular carcinoma (HCC), which is reported to be the fifth most frequently occurring cancer in the world [13, 14]. Epidemiological and animal studies have demonstrated that hepatitis B virus (HBV) and AFM_1 surge the likelihood of HCC in people with hepatitis B surface antigen-positive (HBsAg+) by 3.3-fold [15, 16]. Neuveut et al. [17] asserted that preexisting liver disease due to HBV infection compromises the ability of hepatocytes to debilitate carcinogens such as aflatoxins, thus increasing the chance of HCC.

Regulatory limits are set for countries due to the potential hazards associated with aflatoxin ingestion. In addition to setting regulatory limits for mycotoxins, it is also imperative to conduct health risk assessments on the population due to dietary exposure. Risk evaluation is now widely accepted as the ideal means to assess possible links between hazards in the food chain and actual risks to human health [18].

In Ghana, scanty works have been performed on the prevalence of AFM_1 in this local cheese. This study is unique in Ghana because to the best of our knowledge, it is the only work that investigated the exposure and characterized the risk due to AFM_1 through consuming traditional local soft cheese *wagashie* in different age categories of some regions across Ghana. The risk assessment results could be the scientific basis of risk management options.

2. Materials and Methods

2.1. Wagashie Samples. A total of 182 samples of the *wagashie* samples were randomly bought from local markets of the towns of each region (Twenty-six (26) each), where the Fulanis (Normads) are located in Ghana; Upper West, Upper East, Brong Ahafo, Ashanti, Northern, Eastern, and Greater Accra Regions (Table 1). The *wagashie* samples were collected and stored in sterile specimen containers (Nasco, USA), kept in an ice chest freezer (Thermos 7750, China) with cold packs at a temperature of 2–4°C under aseptic conditions, and transported to the laboratory where they were stored in a refrigerator until they were ready for analysis [19].

2.2. Chemicals and Standards. The analytical standard of AFM_1 was supplied by Sigma-Aldrich (St. Louis, MO, USA). All solvents used for the preparation of the mobile phase were HPLC grade and obtained from Merck (Darmstadt, Germany). All homogenized mixtures and eluates were filtered through Whatman no. 4 and 0.45 mm membrane filters (Whatman plc, Maidstone, UK), respectively. Deionized water was obtained with a Millipore Elix Essential purification system (Bedford, MA, USA). EASI-extracted AFM_1 immunoaffinity columns (stored at 4°C until use) were supplied by R-Biopharm Rhone limited and used for solid-phase extraction (SPE) and clean-up.

2.3. Preparation of Samples. After warming at about 37°C in a water bath, the samples were centrifuged at 2000 g to separate fat layers and then filtered. The prepared test portion of 50 mL was transferred into a syringe barrel attached to the AFM_1 immunoaffinity column and passed at a slow steady flow rate of 1-2 mL/min. The columns were then washed with 20 mL deionized water to wash off unbound particles during clean-up. This was a 4-stage clean-up with water on each stage using 5 ml of deionized water, and the air was passed through the columns to dryness. AFM_1 was eluted with 4 mL pure acetonitrile by allowing it to be in contact with the column for not less than 60 seconds. The eluate was evaporated to dryness using a gentle stream of nitrogen. The residue was dissolved in 500 μL of mobile phase and filtered using a membrane filter (Whatman 0.45 mm membrane filter (Whatman plc, Maidstone, UK) before being injected into HPLC for quantification (CSN EN ISO 14501 IDF 171 : 2007) [20].

2.4. Preparation of Standard Solutions. A stock solution (0.1 μg/mL) was prepared from a standard solution of AFM_1 (0.993 μg/mL in acetonitrile) and stored with care in the freezer. A working stock solution of 0.01 μg/mL was diluted step by step with the combined solution (acetonitrile/water, 75/25, v/v) to prepare a sequence of working solutions that were stored in vials below 4°C for the calibration curve. Calibration solutions of 0.02 μg/kg, 0.04 μg/kg, 0.06 μg/kg, 0.08 μg/kg, and 0.10 μg/kg were used. Samples with AFM_1 amount above the calibration range were diluted, and dilution factors were applied for quantification.

2.5. Instrumentation. Agilent high-performance liquid chromatography system (HPLC 1260 infinity series, OpenLab software, X-bridge column) (250mmx 4.6 mm i.d., 5 μm, USA) with a quaternary pump and fluorescence detection was used for AFM_1 quantification analysis and was carried out as per the method given by EN ISO 14501 : 2007 [20]. Data acquisition and quantification were performed using ChemStation (OpenLab edition). The Agilent HPLC equipped with a fluorescence detector was set at an excitation wavelength of 360 nm and an emission wavelength of 440 nm and the column compartment (HPLC column: TC-C18 (2), 170, 5 μm, 4.6 × 250 mm; thus, a pore size of 170, particle size of 5.0 μm, inner diameter of 4.6 mm, length of

Table 1: Geographical location and some attributes of the origin of *wagashie* samples.

Region	No. of samples	Agro-ecological zones	Rainfall (mm)	Temperature (°C)	Coordinates
Upper West	26/182	Savannah	1000–1200	27.8	10.7082°N, 0.9821°W
Upper East	26/182	Savannah	800–1000	28.3	10.2530°N, 2.1450°W
Brong Ahafo	26/182	Transitional	1400–1600	23.9	7.9559°N, 1.6761°W
Ashanti	26/182	Deciduous forest	1200–1400	26.3	6.7470°N, 1.5209°W
Northern	26/182	Savannah	1000–1200	27.9	9.5439°N, 0.9057°W
Eastern	26/182	Deciduous forest	1400–1900	25.9	6.2374°N, 0.4502°W
Greater Accra	26/182	Coastal savannah	800–1000	26.6	5.8143°N, 0.0747°E

250 mm, and carbon load of 12%) temperature regulated at 35°C. The mobile phase was a mixture of water and acetonitrile at a ratio of 25 : 75 (v/v), respectively, and an isocratic delivery mode was employed at a flow rate of 0.8 ml·min^{-1} with an injection volume of 10 μl.

2.6. Validation. The HPLC-FLD method was validated according to the guidelines of European Commission Decision 657/2002/EC for confirmatory analysis methods, and the tested parameters were linearity, limit of detection (LOD), limit of quantification (LOQ), accuracy, precision, and selectivity. The linearity was assessed by constructing five-point solvent-matched calibrations in triplicate for AFM_1 standard solutions in the concentration range of 0.05 to 0.8 mg/L. Calibration curves were drawn by plotting the peak area against AFM_1 concentration, and linearity was evaluated by linear regression analysis expressed as coefficient of determination (r^2).

The precision of the method was estimated in terms of % RSD of three identical extractions of milk product samples spiked with AFM_1 at the same and at three different spiking levels. Method selectivity was evaluated by analyzing AFM_1 known negative milk product matrix and reagent blank to determine any interference from endogenous substances around the retention time of the target analyte.

2.7. Human Risk Assessment of Exposure to AFM_1 via Consumption of Wagashie

2.7.1. Exposure Estimation. Estimated daily intake (EDI) was considered by using the mean quantity of aflatoxins derived from the *wagashie* samples, the number of samples consumed daily, and the average body weight. The EDI for mean aflatoxin was premeditated according to the following formula (1) and expressed in μg/kg of body weight/day (μg/kg bw/day) [21, 22];

$$\text{EDI} = \frac{\text{daily intake (food)X mean level of AFM1}}{\text{average bodyweight}}. \quad (1)$$

The daily intake of milk products (*wagashie*) in Ghana for adults according to Omore et al. [23] is approximately 0.0137 kg/day (5.0 kg/year).

The different age categories according to EFSA [24] and their corresponding estimated average weights in Ghana used in this study were performed as follows: infants- 2.9 (2.5–3.2) kg [25, 26], toddler -9.8 (7–12.6) kg [27, 28], children -26 (24–28) kg [29, 30], adolescents- 46.25 (38.5–54) kg [31], and adults- 60.7 kg [32].

2.7.2. Human Health Risk Assessment. Genotoxic compounds such as aflatoxins have their risk assessment fittingly computed based on the margin of exposure (MOEs) approach, which was estimated by dividing the benchmark dose lower limit (BMDL) for aflatoxins – 0.2 ng/kg bw/day by toxin exposure [33–35] as expressed in equation (2).

$$\text{MOE} = \frac{\text{Benchmark dose lower limit}}{\text{EDI (Exposure)}}. \quad (2)$$

For AFM_1, TDI was 0.2 ng/kg/day, obtained by dividing TD_{50} (threshold dose per bw) with a variability factor of 5,000. A public health alarm is raised in instances where MOEs are less than 100,000. [35–37].

2.7.3. Estimated Liver Cancer Risk due to Consumption of "Wagshie" Samples. The ingestion of aflatoxins can be linked to the onset of liver cancer [38]. Therefore, liver cancer risk estimation for Ghanaian adult consumers was calculated for aflatoxins [35, 38]. This involved estimating the population cancer risk per 100,000, which is a product of the EDI value and the average hepatocellular carcinoma (HCC) potency figure from individual potencies of hepatitis B surface antigen (HBsAg) (HBsAg-positive and HBsAg-negative groups).

The JECFA [39] estimated potency values for AFM_1, which corresponded to 0.3 cancers/year/100,000 population ng/kg bw/day (uncertainty range: 0.05–0.5) in HBsAg-positive individuals and 0.01 cancers/year/100,000 population/ng/kg bw/day (uncertainty range: 0.002–0.03) in HBsAg-negative individuals [38], that were adopted for this calculation. Moreover, the average HBsAg + prevalence rate of 7.74% (adult-8.36%, 14.3%-adolescents, and 0.55%-children) for Ghana [40] was adopted and 92.26% (100–7.74%) was extrapolated for HBsAg-negative groups. Hence, the average potency for cancer in Ghana was estimated as follows according to equation (3) as prescribed by [35, 38]:

$$\begin{aligned}\text{Average potency} &= [0.03x\ HBsAg - \text{negative individuals in Ghana}] \\ &\quad + [0.01x\ HBsAg - \text{positive individuals/prevalence rate in Ghana}], \\ (0.3 \times 0.077) + (0.01 \times 0.9226) &= 0.0323 \text{ cancers per year per } 100,000 \text{ population per ng aflatoxin/kg bw/day.}\end{aligned} \quad (3)$$

Thus, the cancer risk was estimated using the following formula in equation (4) [35]:

Thus, the population risk was estimated using the following formula in equation (4):

$$\text{Cancer Risk} = \text{Exposure (EDI)} \times \text{Average potency}. \quad (4)$$

2.7.4. Statistical Analysis. The aflatoxin concentrations were calculated using regression analysis from the curves generated from the standards of aflatoxin M_1 with Excel for Microsoft Windows (version 10). One-sample *t*-test was used to compare the means obtained at a 95% confidence interval and 5% level of significance ($p < 0.05$). The statistical results were summarized as median, standard deviation, variance, skewness, standard error of skewness, kurtosis and standard error of kurtosis, and mean values (range from the 25th percentile to the 75th percentile). SPSS 22 (Chicago, USA) was used in the analysis of data. Deterministic risk assessment model calculations for aflatoxins, dietary exposure (estimated dietary intake), MOE values, average potency, and cancer risk were calculated.

3. Results

3.1. AFM1 Concentrations. The limits of detection (LOD) for AFM_1 ranged between 0.13 and 0.15 μg/kg, while limits of quantification (LOQ) ranged between 0.26 and 0.30 μg/kg, respectively, for both.

The summary of statistics for the number of food samples contaminated with AFM_1 is presented in Table 2. The level of occurrence of the AFM_1 ranged between 0 and 3.60 μg/kg, 0 and 2.50 μg/kg, 0 and 3.320 μg/kg, 0 and 2.11 μg/kg, 0 and 2.90, 0–2.55, and 2.90 μg/kg, respectively, for the Upper West, Upper East, Brong Ahafo, Ashanti, Northern, Eastern, and Greater Accra Regions.

The Upper West Region recorded 0.601, 0.070, and 0.971 μg/kg for mean, median, and variance, respectively, while the skewness and kurtosis were 1.812 and 2.695, respectively, and showed that the dataset of aflatoxins (AFM_1) obtained in this town was asymmetrical and heavy tailed (Table 2). The lower and upper limits were 0.204 and 0.4761, respectively, and showed significant differences ($p < 0.05$) (Table 3).

For Upper East, values of 0.741, 0.64, and 0.682 μg/kg were recorded from the summary statistics as mean, median, and variance, respectively, while 0.682 and −0.840 were recorded as skewness and kurtosis and implied moderate skewness and light tailed. The upper and lower limits were 0.407 and 1.074 (Table 2). Values significantly differed ($p < 0.05$) (Table 3).

The mean, median, and variance recorded for Brong Ahafo were 0.907, 0.670, and 0.878 μg/kg, respectively. While the dataset showed was symmetrical and light tailed, the skewness and kurtosis were 0.786 and −0.124, respectively (Table 2). Values of 0.529 and 1.286 were recorded as upper and lower limits (Table 2). There were significant differences ($p < 0.05$) observed (Table 3).

For Ashanti, the mean, median, and variance recorded were 0.725, 0.665, and 0.505 μg/kg, respectively. The dataset for Ashanti was fairly symmetrical and light tailed, and 0.435 and -1.144 for skewness and kurtosis, respectively. Upper and lower limits of 0.438 and 1.024 were, respectively, recorded. There were significant differences ($p < 0.05$) (Tables 2 and 3).

For Northern, the mean, median, and variance recorded were 0.636, 0.000, and 0.921 μg/kg, respectively. The dataset for Northern was fairly symmetrical and light tailed, and 1.119 and −0.273 for skewness and kurtosis, respectively. Upper and lower limits of 0.249 and 1.024 were, respectively, recorded. There were significant differences ($p < 0.05$) (Tables 2 and 3).

In Eastern, the mean, median, and variance recorded were 0.482, 0.000, and 0.634 μg/kg, respectively. The dataset for Eastern was fairly symmetrical and light tailed, and 1.543 and 1.196 for skewness and kurtosis, respectively. Upper and lower limits of 0.159 and 0.803 were, respectively, recorded. There were significant differences ($p < 0.05$) (Tables 2 and 3).

Lastly, for Greater Accra, we recorded mean, median, and variance of 0.504, 0.000, and 0.652 µg/kg, respectively. The dataset for Greater Accra was fairly symmetrical and light tailed, and 1.580 and -1.915 for skewness and kurtosis, respectively. Upper and lower limits of 0.178 and 0.830 were, respectively, recorded. There were significant differences ($p < 0.05$) (Tables 2 and 3).

Regarding the frequency and (percentage %) of positive AFM_1 in contaminated *wagashie* samples, values recorded for overall positive samples were 93/182 (51.1%), while for the different locations, values of 17/26 (65.4%), 14/26 (53.8%), 18/26 (66.7%), 17/26 (65.4%), 9/26 (34.6%), 9/26 (34.6%), and 9/26 (34.6%) for Upper West, Upper East, Brong Ahafo, Ashanti, Northern, Eastern, and Greater Accra regions, respectively, were recorded (Table 4).

3.2. Risk Assessment. The estimated daily intakes (EDIs) of AFM_1 in the *wagashie* samples from the Upper West were 1.42, 0.84, 0.31, 0.18, and 0.14 ng/kg bw/day for infants, toddlers, children, adolescents, and adults, respectively. The margin of exposure (MOE) values recorded were 0.14, 0.24, 0.64, 1.11, and 1.42, respectively. The average potency of the aflatoxins was 0.0323 aflatoxins ng/kg bw/day and produced

Table 2: Summary of statistics of AFM_1 in *wagshie* obtained from four different regions of Ghana.

	Upper West	Upper East	Brong Ahafo	Ashanti	Northern	Eastern	Gt. Accra
No. of samples	26	26	26	26	26	26	26
Mean	0.601	0.741	0.907	0.725	0.636	0.482	0.504
Std. error of mean	0.193	0.162	0.184	0.139	0.188	0.156	0.584
Median	0.070	0.64	0.670	0.665	0.000	0.000	0.000
Std. deviation	0.986	0.826	0.937	0.711	0.959	0.796	0.808
Variance	0.971	0.682	0.878	0.505	0.921	0.634	0.652
Skewness	1.812	0.686	0.786	0.435	1.119	1.543	1.580
Std. error of skewness	0.456	0.456	0.456	0.456	0.456	0.456	0.456
Kurtosis	2.695	−0.840	−0.124	−1.144	−0.273	1.196	1.915
Std. error of kurtosis	0.887	0.887	0.887	0.887	0.887	0.887	0.887
Range	3.600	2.500	3.320	2.11	2.90	2.55	2.90
Percentiles							
25	0.000	0.000	0.000	0.000	0.000	0.000	0.000
50	0.070	0.645	0.670	0.665	0.000	0.000	0.000
75	1.107	1.335	1.817	1.250	1.380	0.830	1.000

Table 3: Statistics of one-sample *t*-test of *wagashie* samples from different regions.

	t	Df	Sig.(2-tailed)	Mean difference	95% confidence interval of the difference	
					Lower	Upper
Upper West	3.114	25	0.005	0.601	0.204	0.999
Upper East	4.577	25	0.000	0.741	0.407	1.074
Brong Ahafo	4.939	25	0.000	0.907	0.529	1.286
Ashanti	5.203	25	0.000	0.725	0.438	1.012
Northern	3.380	25	0.002	0.636	0.249	1.024
Eastern	3.084	25	0.005	0.481	0.159	0.803
Gt. Accra	3.184	25	0.004	0.504	0.178	0.830

Table 4: Proportions of *wagashie* samples that exceeded AFM_1 limits of the European Food Safety Authority (EFSA) and Ghana Standard Authority (GSA).

Region	No. of samples	Exceeding EFSA regulation		Exceeding GSA regulation		Overall positive with AFM_1 <LOD
		Yes (%)	Range	Yes (%)	Range	
Upper West	26	15 (57.7%)	0.06 ± 0.001–3.6 ± 0.7	8 (30.8%)	1.0 ± 0.15–3.61 ± 0.6	17 (65.4%)
Upper East	26	14 (53.8%)	0.55 ± 0.02–2.5 ± 0.7	14 (53.8%)	0.55 ± 0.02–2.5 ± 0.7	14 (53.8%)
Brong Ahafo	26	17 (65.4%)	0.5 ± 0.001–3.3 ± 0.5	16 (61.5%)	0.50 ± 0.03–3.31 ± 0.5	18 (66.7%)
Ashanti	26	17 (65.4%)	0.07 ± 0.001–2.11	15 (57.7%)	0.6–2.11	17 (65.4%)
Northern	26	9 (34.6%)	1.0–2.9	9 (34.6%)	1.0–2.9	9 (34.6%)
Eastern	26	9 (34.6%)	1.1–2.55	8 (38.8%)	1.1–2.55	9 (34.6%)
Gt. Accra	26	9 (34.6%)	1.0 ± 0.001–2.9 ± 0.4	9 (34.6%)	0.61 ± 0.02–2.11 ± 0.4	9 (34.6%)
Total	182	63 (49.5%)	0.06 ± 0.001–3.6 ± 0.5	79 (43.4%)	0.50 ± 0.03–3.6 ± 0.5	93 (51.1%)

European Union Food Safety (EFSA) limit for $AFM_1 = 0.05\,\mu g/kg$ and Ghana Standard Authority (GSA) limit for $AFM_1 = 0.5\,\mu g/kg$.

cancer risks of 0.05, 0.03, 0.01, 5.8×10^{-3}, and 4.52×10^{-3}, respectively (Table 5).

Upper East recorded EDI values of 1.75, 1.04, 0.39, 0.22, and 0.17 ng/kg bw/day for infants, toddlers, children, adolescents, and adults, respectively. MOE values were 0.11, 0.19, 0.51, 0.90, and 1.18. The average potency was the same. Cancer risks of 0.06, 0.03, 0.013, 7.106×10^{-3}, and 5.49×10^{-3}, respectively, for these age categories were recorded (Table 5).

In Brong Ahafo, the EDI values recorded for infants, toddlers, children, adolescents, and adults were 2.14, 1.27, 0.48, 0.27, and 0.20 ng/kg bw/day, respectively. MOE values recorded were 0.09, 0.16, 0.42, 0.74, and 1.00, respectively. The average potency was the same as in other regions, while the cancer risks were 0.069, 0.041, 0.016, 8.72×10^{-3}, and 6.46×10^{-3}, respectively (Table 6).

In Ashanti Region, the EDI values recorded for infants, toddlers, children, adolescents, and adults were 1.71, 1.01, 0.38, 0.21, and 0.16 ng/kg bw/day, respectively. MOE values recorded were 0.12, 0.20, 0.53, 0.95, and 1.25, respectively. The average potency was the same as in other regions, while the cancer risks were 0.06, 0.03, 0.01, 6.78×10^{-3}, and 5.17×10^{-3}, respectively (Table 6).

For Northern Region, the EDI values recorded for infants, toddlers, children, adolescents, and adults were 1.50, 0.89, 0.34, 0.19, and 0.14 ng/kg bw/day, respectively. MOE values recorded were 0.13, 0.22, 0.59, 1.05, and 1.43, respectively. The average potency was the same as in other

Table 5: Risk evaluation for AFM_1 via consumption of *wagashie*.

	Region	Av. body wgt. (kg)	EDI (ng/kg bw/day)	MOE	Cancer risk (cases/100,000 person/yr)
Upper West	Infants (0–11 mths)	2.9	1.42	0.14	0.05
	Toddlers (12–35 mths)	9.8	0.84	0.24	0.03
	Children (36 mths-10 yrs)	26	0.31	0.64	0.01
	Adolescents (11–17 yrs)	46.25	0.18	1.11	5.8×10^{-3}
	Adults (18–64 yrs)	60.7	0.14	1.42	4.52×10^{-3}
Upper East	Infants (0–11 mths)	2.9	1.75	0.11	0.06
	Toddler (12–35 mths)	9.8	1.04	0.19	0.03
	Children (36 mths-11 yrs)	26	0.39	0.51	0.013
	Adolescents (11–17 yrs)	46.25	0.22	0.90	7.106×10^{-3}
	Adults (18–64 yrs)	60.7	0.17	1.18	5.49×10^{-3}

Margin of exposure-MOE. Mean of aflatoxins M1- $U.W = 0.601\,\mu g/kg$, $U.E = 0.741\,\mu g/kg$. Daily intake of wagshie for infants was halved (0.5×0.0137 kg). Average potency of aflatoxin = 0.0323. Average body weights were obtained from the different ranges referenced by the authors. $1\,\mu g = 1000$ ng.

Table 6: Risk evaluation for AFM_1 via consumption of *wagashie*.

	Region	Av. body wgt. (kg)	EDI (ng/kg bw/day)	MOE	Cancer risk (cases/100,000 person/yr)
Brong Ahafo	Infants (0–11 mths)	2.9	2.14	0.09	0.069
	Toddlers (12–35 mths)	9.8	1.27	0.16	0.041
	Children (36 mths-10 yrs)	26	0.48	0.42	0.016
	Adolescents (11–17 yrs)	46.3	0.27	0.74	8.72×10^{-3}
	Adults (18–64 yrs)	60.7	0.20	1.00	6.46×10^{-3}
Ashanti	Infants (0–11 mths)	2.9	1.71	0.12	0.06
	Toddlers (12–35 mths)	9.8	1.01	0.20	0.03
	Children (36 mths-10 yrs)	26	0.38	0.53	0.01
	Adolescents (11–17 yrs)	46.3	0.21	0.95	6.78×10^{-3}
	Adults (18–64 yrs)	60.7	0.16	1.25	5.17×10^{-3}

Margin of exposure-MOE. Means of aflatoxins M1- B.A-0.907 $\mu g/kg$, AR- 0.725 $\mu g/kg$. Daily intake of *wagshie* for infants was halved (0.5×0.0137 kg). Average potency of aflatoxin = 0.0323. Average body weights were obtained from the different ranges referenced by the authors. $1\,\mu g = 1000$ ng.

regions, while the cancer risks were 0.048, 0.029, 0.010, 6.14×10^{-3}, and 4.52×10^{-3}, respectively (Table 7).

Furthermore, the EDI values recorded in Eastern Region for infants, toddlers, children, adolescents, and adults were 1.14, 0.67, 0.25, 0.14, and 0.11 ng/kg bw/day, respectively. MOE values recorded were 0.18, 0.30, 0.53, 1.43, and 1.81, respectively. The average potency was the same as in other regions, while the cancer risks were 0.04, 0.02, 8.08×10^{-3}, 4.52×10^{-3} and 3.55×10^{-3}, respectively (Table 7).

Lastly, for Greater Accra Region, the EDI values recorded for infants, toddlers, children, adolescents, and adults were 1.38, 0.82, 0.31, 0.17, and 0.13 ng/kg bw/day, respectively. MOE values recorded were 0.14, 0.24, 0.64, 1.17, and 1.54, respectively. The average potency was the same as in other regions, while the cancer risks were 0.044, 0.026, 0.01, 5.49×10^{-3}, and 4.20×10^{-3}, respectively (Table 8).

4. Discussion

4.1. AFM_1 Concentrations. All around the globe, consumption of unsafe food has resulted in approximately 420,000 deaths annually and is the cause of more than 200 diseases ranging from diarrhea to cancers [41]. *Wagashie* is consumed by most Ghanaians as a snack to complement and increase the protein content of the predominantly carbohydrate diets to help curb protein malnutrition in Africa [42]. Our results point to comparatively moderate levels of AFM_1 contamination in *wagashie* samples from different locations in Greater Accra Region of Ghana. Our results for mean AFM_1 were in the range of 0.482–0.907 $\mu g/kg$ (482–907 ng/kg), while approximately 93/182 (51.1%) out of the total samples investigated tested positive for AFM_1, which compared satisfactorily well with some published findings of some researchers around the globe.

In the most recent studies related to cheese contamination, greater levels were recorded in other geographical locations, particularly in the Middle East and North Africa where the following AFM_1 incidences and concentration ranges have been reported: 58% and 19–1984 ng/kg in Lebanon [43], 85% and 2–217 ng/kg in Qatar [44, 45], 50% and 19–158 ng/kg in Turkey [46], 39% and 50–2120 ng/kg in Egypt [47], and 100% and 80–5580 ng/kg in Ethiopia [48]. Bakirdere et al. [49] found AFM_1 contamination in 36 of 67 white cheese samples, the levels of which vary between 50 and 2100 ng/kg. In Mexico, an extremely high content of 57%, 1200–5000 ng/kg, was reported by Carvajal-Moreno et al. [50]. In other parts of Africa, some incidences of AFM_1 in cheese have also been reported. In Ghana, Addo-Boadu [51] recorded AFM_1 contamination of 5/23 (21.74%) in *wagashie* samples with levels within a range of 0.006–0.887 $\mu g/kg$ (60–887 ng/kg) in raw milk and milk products sampled from the Greater Accra Region. Milk product samples from urban centers in Kenya contained AFM_1 up to 6,800 ng/L [52]. In Sudan, 95% of milk products were contaminated with AFM_1 ranging between 220 and 6,800 ng/L [53]. Mulunda and Mike [54] reported 100% of

Table 7: Risk evaluation for AFM_1 via consumption of *wagashie*.

	Region	Av. body wgt. (kg)	EDI (ng/kg bw/day)	MOE	Cancer risk (cases/100,000 person/yr)
Northern	Infants (0–11 mths)	2.9	1.50	0.13	0.048
	Toddlers (12–35 mths)	9.8	0.89	0.22	0.029
	Children (36 mths-10 yrs)	26	0.34	0.59	0.010
	Adolescents (11–17 yrs	46.3	0.19	1.05	6.14×10^{-3}
	Adults (18–64 yrs)	60.7	0.14	1.43	4.52×10^{-3}
Eastern	Infants (0–11 mths)	2.9	1.14	0.18	0.04
	Toddlers (12–35 mths)	9.8	0.67	0.30	0.02
	Children (36 mths-10 yrs)	26	0.25	0.53	8.08×10^{-3}
	Adolescents (11–17 yrs)	46.3	0.14	1.43	4.52×10^{-3}
	Adults (18–64 yrs)	60.7	0.11	1.81	3.55×10^{-3}

Margin of exposure-MOE. Means of aflatoxins M1- NR-0.636 μg/kg, ER- 0.482 μg/kg. Daily intake of *wagshie* for infants was halved (0.5 × 0.0137 kg). Average potency of aflatoxin = 0.0323. Average body weights were obtained from the different ranges referenced by the authors. 1 μg = 1000 ng.

Table 8: Risk evaluation for AFM_1 via consumption of *wagashie*.

	Region	Av. body wgt. (kg)	EDI (ng/kg bw/day)	MOE	Cancer risk (cases/100,000 person/yr)
Greater Accra	Infants (0–11 mths)	2.9	1.38	0.14	0.044
	Toddlers (12–35 mths)	9.8	0.82	0.24	0.026
	Children (36 mths-10 yrs)	26	0.31	0.64	0.0100
	Adolescents (11–17 yrs)	46.3	0.17	1.17	5.49×10^{-3}
	Adults (18–64 yrs)	60.7	0.13	1.54	4.20×10^{-3}

Margin of exposure-MOE. Means of aflatoxins M1- GTA-0.584 μg/kg, Daily intake of wagashie for infants was halved (0.5 × 0.0137 kg). Average potency of aflatoxin = 0.0323. Average body weights were obtained from the different ranges referenced by the authors. 1 μg = 1000 ng.

milk product samples contained AFM1 and the levels were within the range of 0.004-0.845 μg/L (4-8450 ng/L). In approximately 74.7% out of 75 cheese samples collected from Minas-Gerais of Brazil, AFM_1 was detected in the range of 20–6920 ng/L, and 20 (26.7%) [55] samples represented levels higher than values earlier reported by Prado et al. [56] in Brazil. In another study, the occurrence of AFM_1 in 177 fresh milk and 40 cheese samples in Kuwait was determined by Dashti et al. [57]. The toxin was recorded in all fresh milk samples except one and 80% of cheese samples. The previously mentioned data revealed a high incidence of AFM_1 in cheese samples.

Moderate contamination levels were obtained in this study and were comparable to the range of values obtained in other studies. In China (100%, 5–235 ng/kg), South Korea (26%, 15–150 ng/kg) [58, 59], and Middle America, represented by El Salvador (92%, 5–485 ng/kg), Nicaragua (82%, 5–415 ng/kg) [60], and Costa Rica (37%, 31–276 ng/kg) [61], the problem of AFM_1 contamination of cheese was less emphasized than in the Middle East Region, except for 54% and 50–309 ng/kg in Iran [62]. In a study conducted by Sharifzadeh et al. [63], 52 of the samples were reported to be contaminated by AFM_1, the levels of which ranged from 50.2 to 424.4 ng/kg. AFM_1 levels of 8 of the samples varied between 250.2 and 424.4 ng/kg. Recently in India, Kaur et al. [64] reported mean values of 321 ng/kg AFM_1 in cheese among other milk product samples.

Nonetheless, lesser quantities compared to our results have been reported in some studies. Ardic et al. [65] reported AFM_1 contamination in 158 of 192 Turkish brined white cheese samples. AFM_1 contamination levels of 51 of the samples exceed 250 ng/kg. In a recent study conducted by Torovic et al. [66] in Serbia, it is shown that 70% of a total of 60 samples were contaminated with AFM_1 and were above 25 ng/kg. Kim-Soo et al. [59] reported an incidence of AFM_1 contamination in 61 cheese samples ranging from 15 to 150 ng/kg (Mean: 37 ng/kg). AFM_1 levels of 25 (65.5%) of total cheese samples are reported to range from 52.2 to 272 ng/kg, with a mean of 158.4 ng/kg by Bahrami et al. [67]. In Fallah's [68] study, toxicity levels of 59 (81.9%) of white cheese samples varied between 30 and 1200 ng/kg (mean: 297 ng/kg). Gurses et al. [69] analyzes 77 cheese samples and reported an incidence of AFM_1 in about 44% of samples; however, none of the AFM_1 levels exceed the legal limit of the TFC. In another study, AFM_1 levels of cheese samples ranged from 16 to 136 ng/kg [70].

It is interesting to note that despite a much higher AFM_1 occurrence rate of 78% recorded in the study by Torovic et al. [66], Serbian cheeses (mean LB 97 ng/kg and UB 103 ng/kg) very closely matched European ones regarding mean content. Other world regions share the same hazard to a different extent. According to a systematic review by [71], the prevalence of AFM_1 in the milk of the Middle East Region during the 1995–2017 period was considerably higher than in Europe.

The contamination rate and levels of AFM_1 in local soft cheese *wagashie* obtained in this study may be because dairy animals kept in local dairy farms were fed with compound rations stored under poor conditions, which can be contaminated with aflatoxins. Hot and humid climatic conditions are very conducive to fungal invasion, growth, and production of mycotoxins including aflatoxins in food and feed commodities [72]. Unseasonal rains and related flash floods are widespread, and this increases the moisture content of the grains and other feedstuff and, therefore, its

vulnerability to fungal attacks. Indeed, several previous findings by some researchers [55, 60, 73] have indicated the presence of high levels of aflatoxins in dairy animals' feed and ingredients from other parts of the world due to seasonal fluctuations and are in agreement with the observation of [66].

Awareness of seasonal fluctuations and their link with the high occurrence of AFM_1 in cheeses can provide the basis for selecting the best season and crop practices that are used for the preparation of feed for livestock, thus preventing the contamination by toxicogenic fungi and the ensuing transfer of AFM_1 to dairy products.

Moreover, most of the dairy farmers prefer to feed cereals (maize and wheat) or agricultural or oilseed byproducts (peanuts and soybean) to their dairy animals, and such aflatoxin-susceptible feed materials constitute more than 70% of cattle feed [72]. Therefore, if such high aflatoxin-contaminated feedstuff is included in the diet of dairy animals, there is always a great possibility of AFM_1 appearing in milk at high levels. Other probable factors, which may play an important role in the high levels of AFM_1 in *wagashie* in this study, include poor farm management practices, especially feed storage practices, no legal limits of aflatoxins exist for livestock feed, and there is a lack of knowledge among dairy farmers concerning aflatoxins.

Aflatoxin exposure early in life has been associated with impaired growth, particularly stunting [74, 75]. Furthermore, early exposure to aflatoxins is a potential risk for synergistic interactions with other toxins as subjects grow [76, 77]. Weaning is a transition period of a child from breast milk to other sources of food, which often results in a marked decrease in nutrient intake in developing countries [78, 79]. One possible variable contributing to poor child health in developing countries is the increased exposure to aflatoxin-contaminated foods following weaning [42, 80].

Gizachew et al. [81] and Skrbic et al. [82] emphasized several factors such as geographical region, season, type and quality of feed, feed storage conditions, and processing methods and conditions that are responsible for the variability of aflatoxin M_1 in milk and dairy products. Lack of fresh forage as feed might have led to a longer storage of hay or feed leading to contamination of *Aspergillus* sp. leading to AFB1 contamination.

4.2. Risk Assessment. Aflatoxins are unaffected by many food processing techniques such as boiling or pasteurization, as they are heat stable [83]. There is always a risk involved with their association with food or feed. Risk estimations as explained by Liu and Wu [84] and Kuiper-Goodman [85] are modeled to predict the magnitude of adverse health implications of mycotoxin exposure and guide food regulators to set thresholds for these toxins in foodstuffs. MOE results obtained in this study implied a high risk for infants, children, and adolescents (total aflatoxins). Our results showed a high risk for cancer due to AFM_1 exposure from *wagashie* consumption for infants, toddlers, children, adolescents, and adults.

Considering the EDI values obtained in a study by Addo-Boadu [51] in Ghana, and contrary to the results of this study, values of 0.124–209 and 0.019–0.034 ng/kg bw/day for infants and adults and elderly were reported that it did not exceed 1 ng/kg bw/day and so indicated a nonserious risk to AFM_1 through raw cow milk product consumption for this age category.

The results of this study are in agreement with earlier studies from Italy where 0.011–0.057 HCC cases/100,000 people in diverse age categories were predicted [86, 87]. Udovicki et al. [73] in their study estimated that the HCC cases depending on the $HBsAg^{+ve}$ prevalence were 0.0036–0.0047 and 0.0007–0.0009 cases/year/10^5 individuals for Serbia and Greece, respectively.

In recent times, Kaur et al. [64] from India also reported a risk of hepatocellular carcinoma (HCC) cases/year/10^5 individuals of different age-groups, which showed that the value of HCC using a deterministic approach was maximum (0.0106) in the age-group "1–9 years" and least (0.0020) in age-group "21–60 years." The hazard index (HI) value found for cheese was 0.34. The health risk assessment indicated that consumers, especially children, in the study area are at higher health risk for AFM_1 due to their low body weight and higher milk products' intake.

The EDI values reported in this study were higher than previous reports in France by Leblanc et al. [88] who reported EDI of 0.02 ng/kg bw/day for AFM_1 through cheese consumption by French adults (15 years and over) and children (3–14 years) [88].

From Serbia, Torovic et al. [66] reported risk estimates, and margins of exposure (MoEs) for AFM_1-induced hepatocellular carcinoma were of low health concern (>100,000) for all population groups, across exposure levels, except for preschool children at high exposure (P95 : 7148). Exclusive consumption of home cheeses resulted in even lower MoEs, indicating a risk for preschool children (P95 : 3867) and children (P95 : 6414). With respect to AFM_1-induced additional hepatocellular cancer cases assessed based on the combined effects of AFM_1 and chronic hepatitis B or/and C viral infection, even the highest estimates were below the level of concern.

Regarding the mean level exposure of adults through cheese, the risk estimates observed in studies from Lebanon [43] and Iran [62], with MoEs at 88,035 and 80,000, respectively, were very similar to the findings of this study. Furthermore, in another comparable study by Guo et al. [89] from China, the mean liver cancer risk (90% CI) connected with AFM1 intake was calculated to be 0.000129 (0.000119–0.000130) cases/year/10^5 individuals at the 99th percentile.

Ahlberg et al. [90] observed the highest risk in some African countries: in Kenya, and MOE was 5000, while in Malawi, a very constricted border of 803 was recorded [15]. Whereas from Chile, Foerster et al. [91] reported that for children, milk products consumed resulted in MOE of 8333 and only 483 in Malawi [15]. Regarding AFM_1-induced extra cancer cases per 100,000 person-years, unique data reported in the study were associated: the Lebanon cheese study reported 0.00038 [43], matching the high end of the risk

range estimated for adults in Vojvodina, Serbia (upper bound potency of AFM_1 and high prevalence of HBV/HCV), whereas approximations based on milk consumed in Kenya were of higher magnitude of 0.004 [90], but still approximately five times lower than data for Malawian adults (0.023) [15].

5. Conclusions

From the findings of this study, it can be deduced that a moderate percentage of 51.1% of traditional soft cottage cheese (*wagashie*) samples collected in different locations of Ghana proved to have AFM_1 contents, and it further showed a public health concern considering the adverse health outcome of the health risk assessments conducted on these *wagashie* samples widely consumed in Ghana since the computed MOE values were less than 100,000.

Nonetheless, regarding the important role of milk, especially dairy products in the human diet, there is substantial concern about the presence of AFM_1 in milk and dairy products. Additional negative health effects of AFM_1 justify its continuous monitoring and update of risk assessment. Hence, it is imperative to use fast methods in the detection of AFM_1 in *wagashie* and milk and dairy products, and the Ghanaian public health authorities have to ceaselessly monitor to detect AFM_1 contamination and need to be suppressed to an ALARA (as low as reasonably achievable) level.

Data Availability

The datasets used during the current study are available from the corresponding author on request.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Authors' Contributions

NKK and NTA designed the study. NKK and TA carried out the experiment, data collection, and analysis and wrote the first version of the manuscript. NKK and TA contributed to the interpretation of the data and critically revised the manuscript. All authors read and approved the final manuscript.

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