

## Research Article

# Microbiological Assessment of Groundnut (*Arachis hypogaea* L.) Sold for Consumption in Ghana

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The postharvest processes of groundnuts often become sources of microbial contamination leading to infections and intoxication. Hence, this study examined the microbial pathogens contaminating groundnuts after harvesting. About 50 samples were randomly collected from four major groundnut-producing towns: Bolgatanga, Chiana, Navrongo, and Bongo, all in the Upper East Region of Northern Ghana, and microbiologically examined using Analytical Profile Index (API® 20E). The results revealed that samples from Bolgatanga were the most contaminated, while Chiana has the least contaminated samples. Several species of bacterial genera such as *Staphylococcus*, *Proteus*, *Escherichia*, *Bacillus*, and *Micrococcus*, and fungal genera including *Aspergillus*, *Fusarium*, *Rhizopus*, *Mucor*, *Saccharomyces*, and *Eurotium* were isolated as the main microbial pathogens contaminating the produce. Navrongo and Bolgatanga recorded the highest rate of bacterial species for unshelled (29.5%) and shelled (30.4%) groundnuts, respectively, while Bongo and Bolgatanga registered the highest rate of fungal species under unshelled (32.8%) and shelled (32.6%) groundnuts, respectively. Due to the high levels of microbial contamination of most of the samples and the kind of microbial species involved, proper hygiene standards must be adopted during the postharvest handling of the shelled and unshelled groundnuts.

## 1. Introduction

Groundnut (*Arachis hypogaea* L.), also known as peanut, is the most widely cultivated leguminous cash crop by the people of Ghana, particularly in the northern regions. Varieties of groundnuts such as Chinese, Mani Pinta, Sinkarrio, and F-Mix are mainly cultivated across the various communities in the country. The cultivation of the Chinese cultivar, in particular, has been a major agricultural activity throughout the communities in the Upper East Region. Tsigbey et al. [1] reported that in a typical farming community in Northern Ghana, about 90% of farm families

engaged in groundnut cultivation. Oteng-Frimpong et al. [2] also found that about 70% of groundnuts produced in Ghana are concentrated in the northern regions of Ghana. The cultivation is carried out on both commercial and subsistence bases. Peanut provides vegetable proteins not only to the people in the northern regions but to the entire Ghanaian community as it is widely consumed in roasted, boiled, and raw forms across the country. Indeed, Bediako et al. [3] and OkşanUçkun [4] reported that peanuts are widely consumed raw, roasted, or boiled or in the form of oil, cookies, flakes, or candies. These studies also showed that a very large percentage of Ghanaians use peanuts or their

products at least once every week. Due to the sufficient protein contents of groundnut, nutritionists have often promoted it as an alternative to animal proteins in Ghana [5].

Despite these benefits, the postharvest processes (packaging, storage, transportation, washing, and personal hygiene) eventually become sources of microbial contamination, which may have adverse health effects on consumers, especially those who often eat it raw. Indeed, poor post-harvest handlings are the major causes of fungal infections and intoxications which may lead to lung infections and cardiovascular diseases [6]. Chang et al. [7] opined that microbial pathogens could cross-contaminate ready-to-eat foods through water, equipment, and poor handling practices by workers. In fact, toxins such as aflatoxin produced in groundnuts are a result of *Aspergillus flavus* contamination. This toxin, in particular, is one of the key hazards to human health and must be avoided at all levels throughout the value chain [8]. A study by Olayinka et al. [9] demonstrated that a large number of harvested peanuts deteriorate due to microbial contamination as a result of poor handling and storage processes. Again, several researchers have reported on specific microbial pathogens associated with groundnuts worldwide. The report of Brar [10] indicated that food-borne disease outbreaks were associated with *Escherichia coli* O157:H7 and *Listeria monocytogenes* contamination of peanuts in developing countries. Little [11] stated that the outbreaks of *Salmonella* contamination of peanuts and their products raised concerns of peanuts being potential vehicles for food-borne disease outbreaks. Zamble et al. [12] also reported the genera *Mucor*, *Alternaria*, *Helminthosporium*, *Geotrichum*, *Fusarium*, *Cladosporium*, *Penicillium*, and *Aspergillus* from groundnuts sampled from markets in Abidjan. Pathogenic molds such as species of *Alternaria*, *Aspergillus*, *Cunninghamella*, *Cladosporium*, *Fusarium*, *Penicillium*, *Rhizopus*, *Trichoderma*, and *Verticillium* have been associated with peanut seeds in several Sub-Saharan African countries [13–16].

In Nigeria, several studies were conducted on microbial assessments of unshelled groundnuts in Benin City, Edo State [17]; unshelled groundnut vended in Yenagoa metropolis, Bayelsa State [18]; roasted groundnut vended in Bauchi State [19]; groundnut sold on highways of Onitsha-Owerri, Southeast Nigeria [20], and groundnuts vended in Aliero Central Market, Kibbi State [21]. However, these studies on groundnuts in their different forms of consumption are yet to be conducted in Ghana. The current study sought to unveil the level of microbial contamination on groundnuts sold for consumption in Ghana.

In Ghana, groundnuts are vended in different forms (i.e., packaged and unpackaged) and state (i.e., shelled and unshelled) on the major streets, in markets and snack shops and grocery stores across the country. Due to these handling conditions of groundnuts, they are often prone to microbial infections and intoxication. Hence, the levels of microbial contamination need to be analyzed to assure the safety of consumers on the groundnuts sold for consumption in the country. This study, therefore, examines the microbiological safety of groundnuts sold for consumption in Ghana.

## 2. Materials and Methods

**2.1. Sample Collection.** Groundnut samples (uncooked shelled and unshelled and cooked unshelled) packaged in white polythene bags for sale were randomly purchased from the street vendors in duplicates. The samples were collected between June and August, 2022. For raw shelled and unshelled groundnuts, five vendors each were selected from the streets of Bolgatanga (10°47'15.15"N and 0°51'28.74"W), Navrongo (10°53'38.49"N and 1°5'31.7292"W), Bongo (10°54'28"N and 0°48'29"W), and Chiana (10°52'0"N and 1°16'0"W) for sample collection. The duplicate samples were put together as one sample from each vendor. Five samples (in duplicates) from each street (town) were labeled as Bolgatanga Sample A–E (BSA-E), Navrongo Sample A–E (NSA-E), Bongo Sample A–E (GSA-E), and Chiana Sample A–E (CSA-E). Ten (10) vendors along the streets of Bolgatanga and Navrongo were randomly sampled, and about 15 g of boiled groundnuts were purchased from each of them, labeled as Vendor A–J. In all, 50 samples were collected and transported with ice to the laboratory for microbial enumeration and characterization. Bolgatanga is the capital city of the Upper East Region, whereas the rest are major towns in the region located in the northern part of Ghana.

### 2.2. Enumeration and Isolation of Microorganisms

**2.2.1. Microbial Enumeration.** The rinse stock and dilution tubes were prepared according to UçkunOkşan and Işıl [22] with little modification. Five (5) grams of each sample were transferred into 95 mL of sterile buffered peptone water (Merck) and shaken vigorously to obtain rinse stocks. After preparing ten-fold serial dilutions, 1 mL each of the dilutions ( $10^{-1}$ – $10^{-6}$ ) were poured and plated using Plate Count Agar (PCA) (OXOID Ltd., Basingstoke, Hampshire, England) and MacConkey Agar (BIOLAB, MERCK) for total viable count (TVC) and total coliform count (TCC), respectively. The plates were then incubated at 37°C for 24 to 48 h. The PCA and MacConkey Agar were supplemented with 2% cycloheximide (Sigma-Aldrich; Steinheim, Germany) to prevent fungal growth. For fungi counts, 100  $\mu$ L of each of the rinse stocks and dilutions were pipetted onto Potato Dextrose Agar (PDA) (MERCK) plates and surface spread until complete diffusion and the plates were subsequently incubated at 25°C for 3 to 8 days. After counting, the representative bacterial and fungal colonies were further subcultured on fresh nutrient agar (NA) (OXOID Ltd., Basingstoke, Hampshire, England) and PDA, respectively, until pure cultures were obtained. Pure cultures obtained were transferred into 20% sterile glycerol and stored at –80°C for not less than six weeks for further analyses.

The method used by UçkunOkşan and Işıl [22] was adopted and modified for the analysis of *Salmonella* spp. The rinse stock was incubated overnight at 37°C. About 100  $\mu$ L and 1000  $\mu$ L of the overnight incubated rinse stocks were transferred into 10 mL of Rappaport Vassiliadis Broth (MERCK) and 100 mL of Selenite Cysteine Broth (MERCK)

and then incubated at 42°C and 37°C, respectively, for 24 to 48 h for selective enrichment step. After incubation, a loopful of each media was streaked on both Xylose Lysine Deoxycholate (XLD) Agar and Hektoen Enteric Agar (MERCK) in triplicates and incubated at 37°C for 24–72 h. The plates were then observed for colony development.

**2.2.2. Microbial Characterization and Identification.** The selected and purified (suspected) bacterial colonies stored in 20% glycerol were subcultured in nutrient agar. They were therefore identified by their carbohydrate fermentation pattern using the Analytical Profile Index (API® 20E) (BioMerieux) and subsequently confirmed using the API database. Fungal morphology on the other hand was carried out using colony features such as shape, size, hyphae, and colour (on agar), while the cell morphology was carried out using the staining procedure reported by Gaddeyaya et al. (2012). Briefly, a portion of the mycelia was cut, placed on the slide, and mixed with 10% potassium hydroxide. Then, a drop of lactophenol cotton blue stain was added; the smear was covered with a cover slide and examined under a digital camera (PentaView 5.0MP, Celestron) fitted with compound lenses.

### 3. Statistical Analysis

Means and standard deviations were determined for microbial counts using Statistical Package for Social Science (SPSS). The means were analyzed using one-way and three-way Analysis of Variance (ANOVA), and a post hoc (Turkey) test was used to compare the means when a significant variation was established by ANOVA at the significance level ( $P < 0.05$ ). Before that, the test of homogeneity of variances was carried out to find out whether or not the assumption of homogeneity of variance was violated.

### 4. Results and Discussion

**4.1. Enumeration of Microflora Associated with Groundnuts.** The results of the enumeration of microbial contaminants of shelled and unshelled raw and unshelled boiled groundnuts sold for consumption in four major communities of the Upper East Region of Ghana are presented in Tables 1 and 2 and Figure 1, respectively. The results indicated that no *Salmonella* representative colony was identified in all the samples analyzed. Al-Moghazy et al. [23] stated that *Salmonella* species in ready-to-eat foods are dangerous to human health; hence, their absence in our samples is great news. Table 1 reveals that out of the five samples collected in duplicates from each of the four major groundnut producing communities, the highest total coliform counts (TCCs), total viable counts (TVCs), and total fungi counts were recorded by CSC (9.30), BSA (9.29), and BSE (7.27) cfu/g for shelled raw groundnuts, and this contamination might be caused during and after shelling. Similarly, for unshelled raw groundnuts, the highest TCC, TVC, and total fungi across the various sampling communities were recorded by CSE (9.09), CSC (8.40), and GSD (5.36) cfu/g under Chiana and Bongo, respectively, and were significantly different from

one another ( $P < 0.05$ ). However, microbial pathogens could contaminate foods through the use of contaminated water and instruments, poor hygiene condition of personnel, and even flies. The high level of microbial contaminations recorded in this study might be as a result of the contaminated water used to wash the groundnuts, unhygienic conditions of the storage containers, packaging material used for selling them, and direct hand contacts by the personnel shelling them. Microbial contamination of food is a public health concern and must be addressed at every point of consumption. According to Suzyemeire et al. [24], a high number of microbes in food might lead to all kinds of food hazards including simple intestine disorders to neurological disorders and even death.

In addition, the mean microbial count of each sample varied significantly between samples and locations ( $P < 0.05$ ). Meanwhile, the overall means of the samples collected at each location revealed that Navrongo presented the safest shelled raw groundnuts ( $3.38 \pm 3.9$ ) with Bolgatanga being the most contaminated ( $5.10 \pm 2.3$ ), while the least mean counts of the unshelled raw groundnuts was registered by Chaian ( $3.07 \pm 2.9$ ) with Bolgatanga recording the highest means counts ( $4.11 \pm 4.3$ ) cfu/g based on the Food and Drug Authority (FDA) (2013) acceptable limits. Most importantly, the overall microbial mean count shown in Figure 1 suggests that the contamination level of the shelled raw groundnuts from all the sampling locations except Navrongo were higher than the unshelled samples. This result was quite alarming since most consumers fail to wash or apply any further treatments to the shelled groundnuts before consumption and may be liable to microbial infections and intoxication. FDA [25] recommended that total viable or aerobic plate counts per gram for nuts and seeds should be  $5 \times 10^3$  cfu/g. This is an indication that the contamination levels of the majority of the samples were beyond the FDA threshold. Hence, shelled raw groundnuts sold for consumption should be subjected to thorough washing with clean and disinfected water before consumption to reduce the microbial load and effects. All the mean counts varied significantly ( $P < 0.05$ ). The high microbial contamination of the shelled raw groundnuts might have originated from the water used for washing, packaging materials, or the bare uncleaned hands used during processing and packaging. The level of contaminations could be reduced significantly if strict good agricultural practices or good manufacturing practices were followed. Codex International Code of Hygiene Practice of nuts indicated that tree nuts must be free from pathogenic contamination [4]. The abovementioned findings favorably agree with the findings of OkşanUçkun [4]. For shelled groundnuts, they specifically indicated that while yeasts and molds recorded counts between 2.7–4.9 log cfu/g and 1.6–3.3 log cfu/g, respectively, the bacterial contamination levels were between 3.0 and 8.7 log cfu/g.

The microbial contamination levels of unshelled boiled groundnuts presented in Table 2 show that except for *Salmonella* count, all the samples collected were contaminated by at least one of the microbial groups measured. Samples J ( $4.1 \pm 70$ ), D ( $5.2 \pm 0.92$ ), and F ( $3.07 \pm 0.90$ )

TABLE 1: Microbial community associated with uncooked shelled and unshelled groundnuts sold for consumption in Ghana (Log<sub>10</sub> cfu/g).

Loc	Shelled raw groundnuts			Unshelled raw groundnuts		
	TCC	TVC	Total fungi	TCC	TVC	Total fungi
<b>Bolgatanga</b>						
BSA	4.41 ± 0.11 <sup>c</sup>	9.29 ± 0.01 <sup>a</sup>	5.98 ± 2.27 <sup>a</sup>	5.23 ± 0.09 <sup>b</sup>	4.98 ± 0.06 <sup>b</sup>	5.00 ± 0.01 <sup>a</sup>
BSB	7.28 ± 0.02 <sup>a</sup>	6.29 ± 0.02 <sup>c</sup>	6.90 ± 0.02 <sup>a</sup>	5.21 ± 0.01 <sup>b</sup>	6.18 ± 0.17 <sup>a</sup>	5.14 ± 0.04 <sup>a</sup>
BSC	6.99 ± 0.02 <sup>a</sup>	7.92 ± 0.02 <sup>b</sup>	5.54 ± 0.03 <sup>a</sup>	6.18 ± 0.16 <sup>a</sup>	6.21 ± 0.21 <sup>a</sup>	4.83 ± 0.15 <sup>b</sup>
BSD	6.87 ± 0.86 <sup>a</sup>	8.13 ± 1.01 <sup>b</sup>	5.82 ± 1.02 <sup>a</sup>	6.06 ± 1.04 <sup>ab</sup>	6.12 ± 0.99 <sup>a</sup>	4.73 ± 0.98 <sup>b</sup>
BSE	5.71 ± 1.10 <sup>b</sup>	6.92 ± 0.01 <sup>c</sup>	7.27 ± 0.27 <sup>a</sup>	6.19 ± 0.01 <sup>a</sup>	4.99 ± 0.01 <sup>b</sup>	4.88 ± 0.01 <sup>a</sup>
<b>Navrongo</b>						
NSA	4.83 ± 1.10 <sup>c</sup>	2.95 ± 1.05 <sup>c</sup>	1.73 ± 0.58 <sup>d</sup>	6.47 ± 0.99 <sup>a</sup>	5.28 ± 1.11 <sup>a</sup>	4.23 ± 0.58 <sup>a</sup>
NSB	6.63 ± 0.64 <sup>a</sup>	0.91 ± 0.11 <sup>d</sup>	0.85 ± 0.05 <sup>d</sup>	6.17 ± 1.00 <sup>a</sup>	6.26 ± 1.04 <sup>a</sup>	4.71 ± 0.54 <sup>a</sup>
NSC	7.29 ± 0.10 <sup>c</sup>	9.19 ± 1.00 <sup>a</sup>	4.10 ± 0.05 <sup>b</sup>	6.41 ± 0.99 <sup>a</sup>	5.92 ± 0.06 <sup>a</sup>	4.22 ± 0.67 <sup>a</sup>
NSD	6.20 ± 0.02 <sup>ab</sup>	2.95 ± 1.06 <sup>c</sup>	2.87 ± 0.10 <sup>c</sup>	5.27 ± 0.92 <sup>ab</sup>	6.34 ± 1.00 <sup>a</sup>	4.63 ± 1.06 <sup>a</sup>
NSE	5.20 ± 1.00 <sup>bc</sup>	4.94 ± 0.06 <sup>b</sup>	6.93 ± 1.04 <sup>a</sup>	4.10 ± 0.02 <sup>b</sup>	5.95 ± 0.05 <sup>a</sup>	4.53 ± 0.96 <sup>a</sup>
<b>Bongo</b>						
GSA	5.39 ± 0.02 <sup>bc</sup>	4.93 ± 0.55 <sup>c</sup>	0.91 ± 0.11 <sup>c</sup>	3.27 ± 0.05 <sup>d</sup>	4.84 ± 1.00 <sup>b</sup>	4.41 ± 0.03 <sup>a</sup>
GSB	4.05 ± 2.04 <sup>c</sup>	7.28 ± 0.00 <sup>b</sup>	2.22 ± 0.39 <sup>b</sup>	2.25 ± 0.03 <sup>e</sup>	7.30 ± 1.00 <sup>a</sup>	0.98 ± 0.08 <sup>b</sup>
GSC	7.28 ± 1.20 <sup>ab</sup>	6.89 ± 0.02 <sup>b</sup>	4.79 ± 0.06 <sup>a</sup>	9.06 ± 0.01 <sup>a</sup>	3.28 ± 0.19 <sup>c</sup>	4.89 ± 0.01 <sup>a</sup>
GSD	2.89 ± 1.96 <sup>c</sup>	6.70 ± 0.24 <sup>b</sup>	1.38 ± 0.98 <sup>bc</sup>	6.00 ± 0.01 <sup>b</sup>	5.38 ± 0.11 <sup>b</sup>	5.36 ± 2.01 <sup>a</sup>
GSE	9.29 ± 0.29 <sup>a</sup>	8.59 ± 0.61 <sup>a</sup>	4.05 ± 0.06 <sup>a</sup>	4.79 ± 0.01 <sup>c</sup>	2.69 ± 1.00 <sup>c</sup>	3.74 ± 0.02 <sup>a</sup>
<b>Chiana</b>						
CSA	8.41 ± 0.04 <sup>a</sup>	8.30 ± 1.00 <sup>a</sup>	0.86 ± 0.16 <sup>d</sup>	8.08 ± 1.01 <sup>ab</sup>	2.08 ± 1.00 <sup>c</sup>	1.49 ± 0.70 <sup>b</sup>
CSB	5.04 ± 0.95 <sup>b</sup>	4.82 ± 0.12 <sup>b</sup>	3.19 ± 0.01 <sup>b</sup>	7.40 ± 1.01 <sup>b</sup>	3.39 ± 1.01 <sup>bc</sup>	0.94 ± 0.04 <sup>b</sup>
CSC	9.30 ± 0.30 <sup>a</sup>	7.29 ± 0.01 <sup>a</sup>	3.71 ± 0.03 <sup>a</sup>	3.96 ± 1.05 <sup>c</sup>	8.40 ± 1.01 <sup>a</sup>	2.10 ± 1.00 <sup>ab</sup>
CSD	3.90 ± 1.00 <sup>b</sup>	5.20 ± 1.00 <sup>b</sup>	2.89 ± 0.01 <sup>c</sup>	1.96 ± 1.00 <sup>d</sup>	4.94 ± 1.96 <sup>b</sup>	3.07 ± 1.00 <sup>a</sup>
CSE	4.81 ± 1.00 <sup>b</sup>	4.92 ± 0.02 <sup>b</sup>	0.85 ± 0.03 <sup>d</sup>	9.09 ± 0.01 <sup>a</sup>	2.97 ± 1.02 <sup>bc</sup>	1.95 ± 1.05 <sup>ab</sup>

<sup>1</sup>Values are means of triplicate determinations from three independent trials; ± = standard deviation; TCC = total coliform count; TVC = total viable counts; BS = Bolgatanga sample; NS = Navrongo sample; GS = Bongo sample; CS = Chiana sample. <sup>2</sup>Means with the same letter as superscript in a column are significantly different ( $P < 0.05$ ).

TABLE 2: Microbiology of unshelled boiled groundnuts sold for consumption (Log<sub>10</sub> cfu/g).

Location	TCC	TVC	Fungi	Mean ± SD
A	2.10 ± 1.00 <sup>bc</sup>	4.20 ± 1.00 <sup>a</sup>	ND	1.60 ± 0.60 <sup>a</sup>
B	1.30 ± 1.00 <sup>c</sup>	4.00 ± 0.50 <sup>a</sup>	ND	1.30 ± 0.30 <sup>ab</sup>
C	1.40 ± 1.31 <sup>c</sup>	2.40 ± 1.25 <sup>b</sup>	1.40 ± 0.40 <sup>c</sup>	1.60 ± 0.40 <sup>a</sup>
D	ND	5.20 ± 0.92 <sup>a</sup>	ND	1.30 ± 0.30 <sup>ab</sup>
E	ND	1.50 ± 1.00 <sup>b</sup>	ND	0.40 ± 0.00 <sup>c</sup>
F	ND	2.40 ± 0.60 <sup>b</sup>	3.07 ± 0.90 <sup>a</sup>	1.40 ± 0.40 <sup>a</sup>
G	ND	ND	1.90 ± 0.30 <sup>bc</sup>	0.53 ± 0.25 <sup>c</sup>
H	ND	ND	2.60 ± 1.00 <sup>ab</sup>	0.70 ± 0.00 <sup>c</sup>
I	2.90 ± 0.10 <sup>b</sup>	ND	ND	0.77 ± 0.23 <sup>bc</sup>
J	4.10 ± 1.00 <sup>a</sup>	ND	1.90 ± 1.00 <sup>bc</sup>	1.50 ± 0.50 <sup>a</sup>

<sup>1</sup>Values are means of triplicate determinations from three independent trials; ± = standard deviation; ND = not determined; TCC = total coliform count; TVC = total viable counts; BS = Bolgatanga sample; NS = Navrongo sample; GS = Bongo sample; CS = Chiana sample. <sup>2</sup>Means with the same letters as superscripts in a column are significantly different ( $P < 0.05$ ).

registered the highest TCC, TVC, and fungal counts, respectively. However, samples E ( $0.4 \pm 0.00$ ) and G ( $0.53 \pm 0.25$ ) were found to be the least contaminated boiled groundnuts with samples A ( $1.6 \pm 0.60$ ) and C ( $1.6 \pm 0.60$ ) being the most contaminated boiled groundnuts. Food and Drug Authority (FDA) recommended that the *Salmonella* counts on fruits and vegetables, tree nuts, and seeds should be zero per 25 g of sample and  $10^2$  cfu/g for total fungal counts [25]. This means that all the samples were safe for *Salmonella* and fungi.

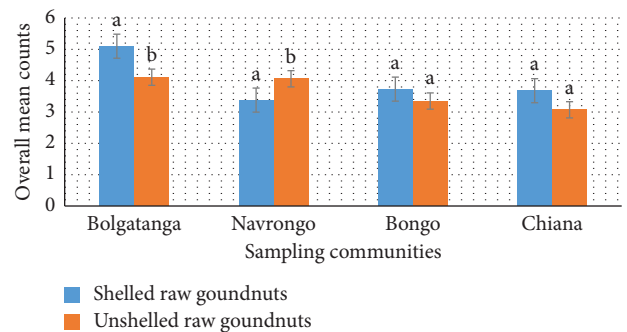


FIGURE 1: Comparison of the level of microbial contamination of both shelled and unshelled groundnuts. Note: bars with the same letters are not significantly different at  $P \geq 0.05$ ; data presented are mean ± SD of triplicates of independent experiments.

## 4.2. Characterization and Identification of Microbial Community Associated with Groundnuts

**4.2.1. Bacterial Isolates.** Five (5) bacterial genera and 18 species were identified from the groundnut samples across the sampling communities. These genera include *Staphylococcus* (*S. aureus*, *S. saprophyticus*, and *S. epidermidis*), *Proteus* (*P. vulgaris*, *P. myxofaciens*, *P. hauseri*, *P. mirabilis*, and *P. penneri*), *Escherichia* (*E. coli* O157), *Bacillus* (*Bacillus cereus*, *B. subtilis*, *B. licheniformis*, *B. larvae*, *B. lentimorbus*, *B. popilliae*, and *B. sphaericus*), and *Micrococcus* (*M. roseus* and *M. luteus*). Table 3 shows the bacterial contaminant

TABLE 3: Bacterial contaminant levels (%) of unshelled and shelled raw groundnuts sampled from the four major communities.

Location	<i>Bacillus</i> spp.		<i>Micrococcus</i>		<i>S. aureus</i>		<i>E. coli</i>		<i>Proteus</i> spp.		% total	
	U	S	U	S	U	S	U	S	U	S	U	S
Bolgatanga (%)	<b>30.0</b>	<b>30.4</b>	<b>21.2</b>	<b>27.8</b>	<b>25.8</b>	<b>26.1</b>	<b>19.0</b>	<b>52.9</b>	<b>64.3</b>	<b>38.1</b>	<b>28.8</b>	<b>30.4</b>
BSA	2	—	—	3	3	1	2	—	2	—	—	—
BSB	4	—	3	—	1	—	—	1	5	2	—	—
BSC	3	3	1	—	2	2	—	3	1	1	—	—
BSD	1	—	—	2	2	2	2	—	2	1	—	—
BSE	2	4	3	—	—	1	—	—	1	2	—	—
Navrongo (%)	<b>22.5</b>	<b>17.4</b>	<b>33.3</b>	<b>33.3</b>	<b>51.6</b>	<b>39.1</b>	<b>14.3</b>	<b>17.6</b>	<b>14.3</b>	<b>19.0</b>	<b>29.5</b>	<b>25.5</b>
NSA	3	—	—	2	6	2	1	—	—	2	—	—
NSB	—	—	—	—	3	4	—	—	—	2	—	—
NSC	—	—	6	—	4	1	—	—	—	—	—	—
NSD	4	1	1	—	—	1	—	1	—	—	—	—
NSE	2	3	4	4	3	1	2	2	2	—	—	—
Bongo (%)	<b>12.5</b>	<b>26.1</b>	<b>24.2</b>	<b>16.7</b>	<b>12.9</b>	<b>21.7</b>	<b>42.9</b>	<b>11.8</b>	<b>0.0</b>	<b>23.8</b>	<b>18.7</b>	<b>20.6</b>
GSA	2	1	—	—	3	1	2	—	—	2	—	—
GSB	—	1	—	—	1	1	3	—	—	—	—	—
GSC	—	1	4	—	—	1	—	—	—	—	—	—
GSD	—	2	1	1	—	2	3	1	—	—	—	—
GSE	3	1	3	2	—	—	1	1	—	3	—	—
Chiana (%)	<b>35.0</b>	<b>26.1</b>	<b>21.2</b>	<b>22.2</b>	<b>9.7</b>	<b>13.0</b>	<b>23.8</b>	<b>41.2</b>	<b>21.4</b>	<b>19.0</b>	<b>23.0</b>	<b>23.5</b>
CSA	4	1	4	—	—	1	1	2	2	1	—	—
CSB	4	1	—	—	—	1	1	—	—	—	—	—
CSC	6	2	—	2	3	—	—	2	—	—	—	—
CSD	—	—	3	2	—	—	—	1	1	—	—	—
CSE	—	2	—	—	—	1	3	2	0	3	—	—
Total	<b>40</b>	<b>23</b>	<b>33</b>	<b>18</b>	<b>31</b>	<b>23</b>	<b>21</b>	<b>17</b>	<b>14</b>	<b>21</b>	<b>139</b>	<b>103</b>

BS = Bolgatanga sample; NS = Navrongo sample; GS = Bongo sample; CS = Chiana sample; U = unshelled; S = shelled.

levels of unshelled and shelled raw groundnuts sampled from the four major groundnut-producing communities. *Bacillus* spp. contamination of unshelled groundnut was highest in samples from Chiana (35%) and lowest in Bongo samples (12.5%). Unshelled groundnut from Navrongo had the highest *Micrococcus* contamination of 33.3% with Bolga and Chiana each having the least (21.2%). *S. aureus* contamination of unshelled groundnut was highest in samples from Navrongo (51.6%) and lowest in samples from Chiana (9.7%). Bongo had the highest (42.9%) *E. coli* contamination of unshelled groundnut samples with Navrongo having the least (14.3%). Contamination of unshelled groundnut by *Proteus* spp. was highest in samples from Bolga (64.3%) but not observed at all in samples from Bongo. Shelled groundnut contamination by *Bacillus* spp. was highest in Bolga (30.4%) and lowest in Navrongo (17.4%). *Micrococcus* contamination of shelled groundnut from Navrongo was highest (33.3%), while samples from Bongo showed the least contamination (16.7%). Samples of shelled groundnut from Navrongo were the most contaminated (39.1%) by *S. aureus* with samples from Chiana being the least contaminated (13.0%). Bolgatanga had shelled groundnut samples with the highest *E. coli* contamination of 52.9%, while Bongo had the lowest of 11.8%. The highest (38.1%) and lowest (19.0%) *Proteus* spp. contaminant levels were recorded for samples taken from Bolgatanga and Navrongo, respectively. Suzy-meire et al. [24] indicated that the bacteria are the main microbial groups causing food disorders. They also added that, as a result of their diversity and pathogenesis, they are

by far the most important microbial group linked to food-transmitted diseases.

A similar study by Song and Kang [26] indicated that the level of *E. coli* and *Staphylococcus* species in 148 peanut samples purchased from snack shops in Brazil were beyond acceptable limits. With the rate of the overall bacterial community associated with both shelled and unshelled raw groundnuts, the shelled raw groundnuts from Bolgatanga were the most contaminated (30.4%), while the highest contamination rate for unshelled groundnuts was registered by Navrongo (29.5%) (Figure 2). This result was not surprising because samples from both Bolgatanga and Navrongo possessed the highest rates of individual bacterial contamination. Further analysis also revealed that *Bacillus* 40 (28.8%) and *Micrococcus* 33 (23.7%) species were the most predominant bacterial contaminants of unshelled raw groundnut and *Bacillus* 23 (22.3%) and *Staphylococcus* 23 (22.3%) species for shelled raw groundnuts from the four locations (Figure 3).

In similar studies, while Beuchat et al. [27] reported that *Bacillus cereus*, *Clostridium botulinum*, *Clostridium perfringens*, *Cronobacter*, *Escherichia coli* O157:H7, *L. monocytogenes*, and *Staphylococcus aureus* were the dominant food-borne bacterial pathogens infecting tree nuts and groundnuts, Al-Moghazy et al. [23] also reported *Streptococcus*, *Staphylococcus*, *Bacillus*, *Xanthomonas*, *Achromobacter*, *Pseudomonas*, *Micrococcus*, and *Brevibacterium* as the main bacterial pathogens found on the surfaces of tree nuts.

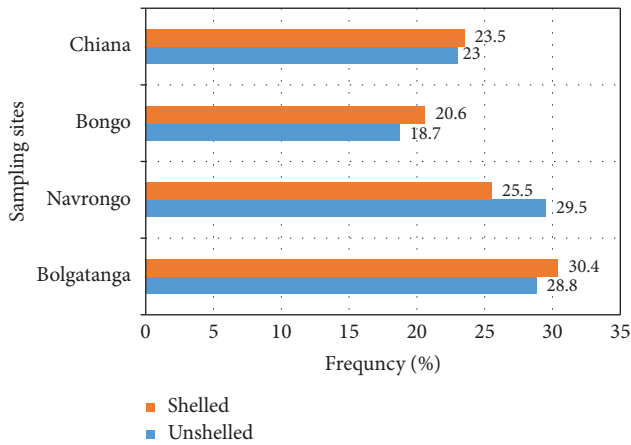


FIGURE 2: The rate of overall bacterial community associated with both shelled and unshelled raw groundnuts.

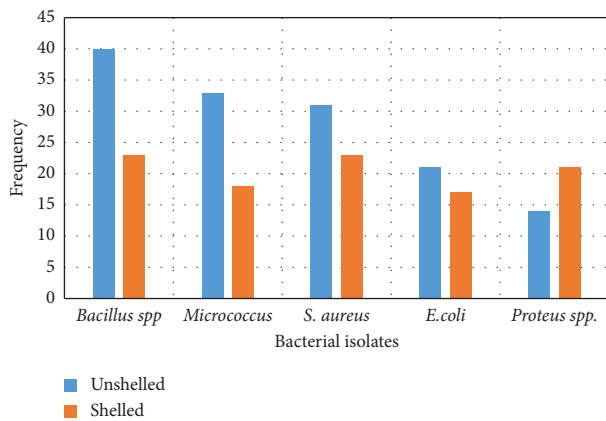


FIGURE 3: Bacterial species associated with both shelled and unshelled raw groundnuts.

**4.2.2. Fungal Isolates.** All the fungal isolates proliferated very well at a temperature of 25°C for 5–7 days. They all have different pH and NaCl tolerances (Table 4). The colonial characteristics, which also helped in the identification, were shape, elevation, colour, optical density, surface, and edge (Table 5). The fungal isolates identified were *Rhizopus* spp., *Aspergillus* spp., *Fusarium* spp., *Mucor* spp., *Eurotium* spp., and *S. cerevisiae* (Table 6). *S. cerevisiae* colonies exhibited regular shapes, entire margins, and glistening surfaces which were different from the other fungal isolates. All the isolates have malty odour and different colony colours. Selected stained and wet-mount fungi cells under the microscope are presented in Figure 4. *Mucor* spp. has brown spores inside spherical sporangia at the tips of the *sporangiophores* with large dark *zygospores*, while *Rhizopus* spp. produced dark, spherical sporangia containing dark to pale spores and large columellate. *Fusarium* spp. produced two types of *macroconidia*. Some are borne on mycelia and are spindle-shaped, straight to slightly curve. *Aspergillus* was recognized by its distinct conidiophores arising from well-defined “foot cells” and terminated by swollen vesicle-bearing flask-shaped *phialides*. The spores are produced in long chains from the ends of the *phialides*.

According to Pitt and Hocking [28] and Horn [29], freshly harvested shelled and unshelled peanuts may be colonized by the diversity of fungi. Although the shell represents a physical barrier and protects the seeds from fungal invasion, fungi may still enter via cracks in the shells. Again, an extensive study was carried out by Ismail [30] in Uganda and Kenya on peanuts reported that the most frequently isolated species was *Aspergillus*, followed by *Macrophomina phaseolina*, and species of *Eurotium*, *Rhizopus*, *Fusarium*, and *Penicillium chrysogenum* were also common. Ihejirika et al. [31], Oluma and Nwankiti [32], and Barros et al. [33] have also reported a similar range of fungi in stored peanuts. *Mucor* spp. has also been isolated from hazelnuts, soybeans, and peanuts [34, 35].

Table 6 shows the distribution of fungal isolates identified from both shelled and unshelled raw groundnuts. Out of 128 fungal species identified from unshelled raw groundnuts sold for consumption, *Aspergillus* species recorded the highest frequency (30) followed by *Rhizopus* and *Fusarium* species (28 each). Again, out of the 92 fungal isolates identified from shelled raw groundnuts, *Rhizopus* and *Mucor* species recorded the highest frequencies (19 each). This level of fungal contamination may present a health risk to consumers since these fungi produce different kinds of toxins of different toxicity levels. Bediako et al. [3] and Torres et al. [36] reported that the high rate of *Aspergillus* species, particularly *A. flavus*, indicated high contamination of aflatoxin. The overall highest rates of fungi contamination were presented by Bongo for unshelled groundnuts (32.8%) and Bolgatanga for shelled raw groundnuts (32.6%) (Figure 5). All the sampling locations registered at least one fungal contaminant of shelled groundnuts except Navrongo which had *Mucor* spp. as an additional contaminant. The contamination could be caused by the water used for washing, bare hands used during washing and packaging, and packaging and storage materials. Mensah et al. [37] pointed that the variation and the level of contaminations could be caused by the environment in which they were found and the unhygienic conditions of persons involved in the vending.

The current findings are in line with several similar studies. In Ghana, Bediako et al. [3] reported that out of 1,237 fungal isolates identified from stored groundnuts, *Aspergillus* species accounted for about 66%. Elsewhere, Tobin-West et al. [6] reported species of *Aspergillus*, *Rhizopus*, *Penicillium*, *Mucor*, and *Fusarium* as the predominant fungi isolated from groundnut samples purchased from four different markets in Port Harcourt, Nigeria, whereas Kigigha et al. [18] identified species of *Aspergillus*, *Penicillium*, *Fusarium*, *Mucor*, and *Rhizopus* from unshelled groundnut purchased from Yenagoa metropolis of Nigeria, and Akinnibosun and Osawaru [17] reported *Aspergillus niger*, *Aspergillus flavus*, *Mucor* spp., *Rhizopus* spp., *Penicillium* spp., *Trichoderma* spp., and *Fusarium* spp. as the predominant fungal species isolated from shelled and unshelled groundnut bought from Benin City. Comparatively, the current study revealed that *Aspergillus* was the predominant fungal species found in the 20 unshelled samples and *Rhizopus* spp. and *Mucor* spp. in the 20 shelled samples

TABLE 4: Preliminary identification of fungi associated with groundnuts sold for consumption in the Upper East Region.

Fungi	T (°C)	NaCl (%)	CD (mm)	pH	PB	PS	PSG	PC	PSP	PR	PST
<i>Rhizopus</i> spp.	25	15	20–55	5.5	–	+	+	–	–	+	+
<i>Saccharomyces</i>	25	7.5	5–15	7.0	+	+	–	–	–	–	–
<i>Aspergillus</i> spp.	25	5.5	22–30	6.0	–	–	–	+	+	–	–
<i>Fusarium</i> spp.	25	25	50–60	7.5	–	+	+	–	+	+	–
<i>Mucor</i> spp.	25	15	25–40	4.5	–	+	+	–	–	–	–
<i>Eurotium</i> spp.	25	25	15–25	5.0	–	+	–	+	+	–	–

+: present; –: absent; P: presence of; B: budding; S: spores; SG: sporangia; C: conidia; SP: septa; and R: rhizoids.

TABLE 5: Morphological characteristics of fungal mycelia isolates from groundnuts sold for consumption in the Upper East Region.

Organism	Colour	Shape	Edge	Elevation	Surface
<i>Rhizopus</i> spp.	WPG	Irregular	Rough	Raised	Fine
<i>S. cerevisiae</i>	Off-white	Regular	Entire	Raised	Glistening
<i>Aspergillus</i> spp.	Grayish yellow	Irregular	Rough	Raised	Coarse
<i>Fusarium</i> spp.	WPV	Irregular	Rough	Raised	Coarse
<i>Mucor</i> spp.	Pale brown	Irregular	Rough	Raised	Coarse
<i>Eurotium</i> spp.	Orange	Irregular	Rough	Raised	Coarse

WPG: white to pale grey; WPV: white to pale violet.

TABLE 6: Distribution of fungal isolates associated with shelled and unshelled raw groundnuts sold for consumption (%).

Location	<i>Rhizopus</i>		<i>S. cerevisiae</i>		<i>Aspergillus</i>		<i>Fusarium</i>		<i>Mucor</i>		<i>Eurotium</i>		% total	
	U	S	U	S	U	S	U	S	U	S	U	S	U	S
Bolga (%)	<b>25.0</b>	<b>47.4</b>	<b>18.8</b>	<b>30.8</b>	<b>40.0</b>	<b>38.9</b>	<b>25.0</b>	<b>25.0</b>	<b>27.3</b>	<b>36.8</b>	<b>6.7</b>	<b>0.0</b>	<b>25.8</b>	<b>32.6</b>
BSA	1	2	3	—	—	—	1	1	—	3	—	—	—	—
BSB	1	—	—	—	7	2	3	—	2	2	—	—	—	—
BSC	3	2	—	—	1	3	—	—	1	—	—	—	—	—
BSD	2	3	—	1	3	2	—	—	—	2	1	—	—	—
BSE	—	2	—	3	1	—	3	2	—	—	—	—	—	—
Navrongo (%)	<b>14.3</b>	<b>10.5</b>	<b>37.5</b>	<b>23.1</b>	<b>20.0</b>	<b>22.2</b>	<b>32.1</b>	<b>0.0</b>	<b>18.2</b>	<b>26.3</b>	<b>33.3</b>	<b>27.3</b>	<b>25.0</b>	<b>18.5</b>
NSA	—	2	3	1	—	1	3	—	—	1	1	—	—	—
NSB	—	—	2	—	—	1	2	—	—	3	1	—	—	—
NSC	3	—	—	—	4	—	4	—	—	—	—	—	—	—
NSD	—	—	1	—	1	—	—	—	—	—	—	—	—	—
NSE	1	—	—	2	1	2	—	—	2	1	3	3	—	—
Bongo (%)	<b>35.7</b>	<b>26.3</b>	<b>43.8</b>	<b>15.4</b>	<b>33.3</b>	<b>33.3</b>	<b>10.7</b>	<b>50.0</b>	<b>45.5</b>	<b>15.8</b>	<b>46.7</b>	<b>45.5</b>	<b>32.8</b>	<b>29.3</b>
GSA	2	2	2	—	3	1	—	3	2	1	4	2	—	—
GSB	1	—	2	—	1	2	—	—	—	1	1	—	—	—
GSC	4	—	1	—	2	—	3	—	—	1	1	—	—	—
GSD	1	—	1	—	1	—	—	1	1	—	—	—	—	—
GSE	2	3	1	2	3	3	—	2	2	—	1	3	—	—
Chiana (%)	<b>25.0</b>	<b>15.8</b>	<b>0.0</b>	<b>30.8</b>	<b>6.7</b>	<b>5.6</b>	<b>32.1</b>	<b>25.0</b>	<b>9.1</b>	<b>21.1</b>	<b>13.3</b>	<b>27.3</b>	<b>16.4</b>	<b>19.6</b>
CSA	2	—	—	2	—	—	1	1	—	2	1	1	—	—
CSB	2	—	—	—	—	—	1	—	—	2	—	—	—	—
CSC	—	—	—	—	2	—	4	—	—	—	—	—	—	—
CSD	—	—	—	—	—	—	2	—	1	—	—	—	—	—
CSE	3	3	—	2	—	1	1	2	—	—	1	2	—	—
Total	<b>28</b>	<b>19</b>	<b>16</b>	<b>13</b>	<b>30</b>	<b>18</b>	<b>28</b>	<b>12</b>	<b>11</b>	<b>19</b>	<b>15</b>	<b>11</b>	<b>128</b>	<b>92</b>

Key: BS = Bolgatanga sample; NS = Navrongo sample; GS = Bongo sample; CS = Chiana sample; U = unshelled; S = shelled.

examined, while Tobin-West et al. [6], Akinnibosun and Osawaru [17], and Kigigha et al. [18] reported that *Aspergillus* spp. predominated both shelled and unshelled groundnut samples sold for consumption.

Furthermore, work on the microbial contamination levels of boiled groundnuts sold for consumption revealed two species each of *Klebsiella* and *Staphylococcus* as well as *Rhizopus* and *Aspergillus*. Table 7 shows



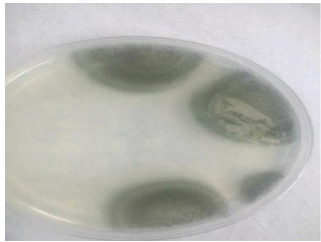
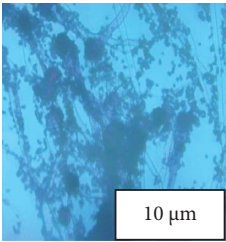

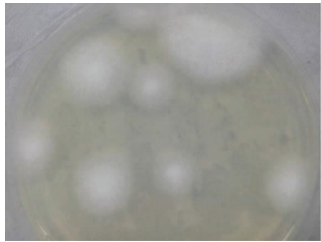
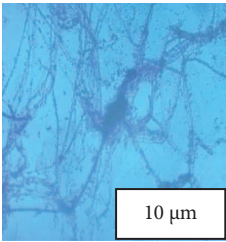
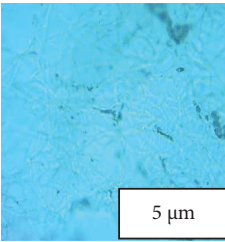

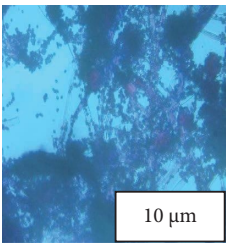
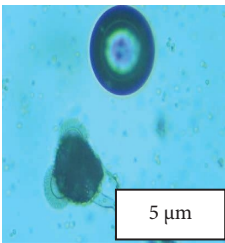

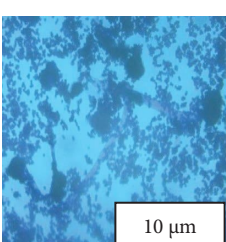
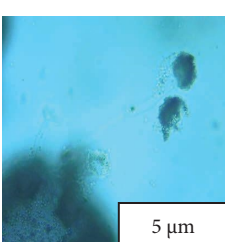

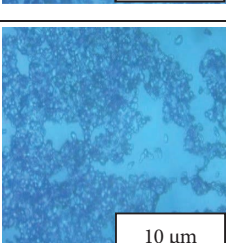
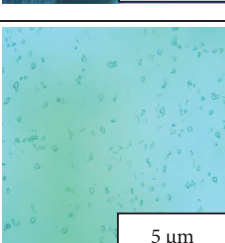

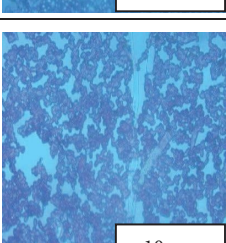
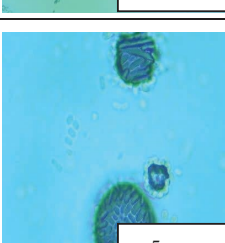
Isolate	Colony morphology	Microscopy-staining/wet-mount		Fungi identity
BSA, B, E NSB, C, E GSB, E CSA-E				<i>Aspergillus</i>
BSA, B, C, E; GSB, D, E; CSA, C, E				<i>Fusarium</i>
BSA, C, D, E; NSA, B, C, D; GSA, D, E; CSD, E				<i>Rhizopus</i>
BSC, BSE				<i>Mucor</i>
BSA-E, NSA-E				<i>S. cerevisiae</i>
BSD, BSE, NSA				<i>Eurotium</i>

FIGURE 4: Colony and microscopic morphology of fungi isolated from groundnuts.



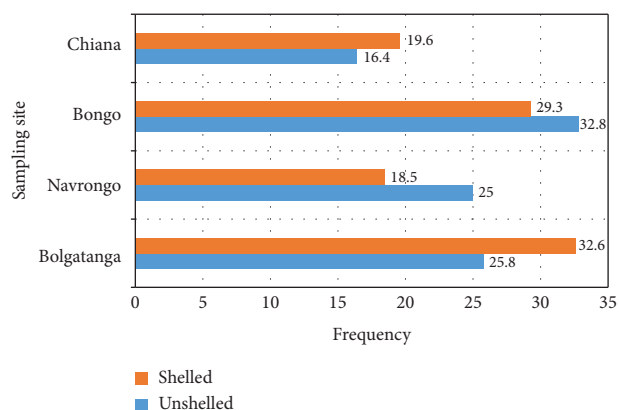


FIGURE 5: The rate of overall fungal community associated with both shelled and unshelled raw groundnuts.

TABLE 7: The microbial community associated with unshelled boiled groundnuts sold for consumption in Bolgatanga and Navrongo township.

Samples	Bacterial isolates		Fungal isolates	
	<i>Klebsiella</i>	<i>Streptococcus</i>	<i>Rhizopus</i> spp.	<i>Aspergillus</i>
A	1	—	—	—
B	—	—	—	—
C	3	—	1	2
D	—	—	—	2
E	1	3	—	—
F	—	—	—	1
G	—	—	2	—
H	—	—	—	1
I	2	1	—	—
J	1	4	3	2
Total	8	8	6	8

a total of 16 bacterial and 14 fungal isolates identified with at least one isolate associated with the ten samples analyzed.

## 5. Conclusion

The bacterial species identified from the groundnut samples from the four major groundnut producing communities in the Upper East Region of Ghana were *Staphylococcus* (4), *Proteus* (5), *Escherichia coli* O157 (1), *Bacillus* (7), and *Micrococcus* (2). Fungal species identified include *Aspergillus*, *Fusarium*, *Rhizopus*, *Mucor*, *S. cerevisiae*, and *Eurotium*. The absence of *Salmonella* in the samples was good news to the consumers. However, even though Navrongo recorded the least overall mean count for shelled raw groundnut and Chiana for unshelled raw counts, some individual samples were highly contaminated as compared to FDA [25] recommendation for fecal coliform count ( $10^2$  cfu/ml), aerobic plate counts ( $10^3$  cfu/ml), and yeast and mold count ( $10^2$  cfu/ml). The high levels of bacterial and fungal contaminants on the samples are quite alarming, and hygienic steps must be taken by vendors to reduce or avoid the rate of contamination. Personal hygiene, market sanitation, and awareness creation on good storage practices

should be carried out among the vendors from the study locations for the improvement of the quality of raw and processed groundnuts sold for consumption.

## Data Availability

The data used to study the findings of this study are included within the article.

## Additional Points

Highlights. (i) Several species of bacteria and fungi were identified from the groundnut samples, (ii) the most occurring bacterium is *Bacillus*, followed by *Micrococcus*, (iii) another most occurring bacterium is *Aspergillus*, followed by *Fusarium*, (iv) samples from Bolgatanga were the most contaminated with bacteria and fungi, and (v) shelled raw groundnuts from Navrongo and unshelled raw groundnuts from Chiana were the safest.

## Conflicts of Interest

The authors declare that they have no conflicts of interest.

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