

## Research Article

# Microbial Quality of Some Fresh-Cut Ready-to-Eat Fruits Sold in Some Markets and Bus Terminals in the Kumasi Metropolis, Ghana

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The consumption of fruits is an important source of vitamins and micronutrients. Despite their health benefits, fruits have been linked to food-borne infections, particularly when untreated animal excreta are used in their cultivation and not properly washed before consumption. This study aimed to evaluate the parasitological, fungal, and bacteriological quality of some fresh-cut readyto-eat fruits sold in markets and bus terminals in the Kumasi Metropolis, Ghana. A total of 270 fresh-cut ready-to-eat fruits, including pineapples, watermelon, pawpaw, sugarcane, and tiger nuts, were sampled from both stationary vendors and hawkers from six different locations in the Kumasi Metropolis. Samples were analysed for the presence of bacteria, fungi, and intestinal parasites using the Microbial Methods in the Bacteriological Analytical Manual (BAM) of the United States Food and Drugs Administration (2001). The pour plate technique was used to culture and grow bacteria and fungi, while the most probable number (MPN) table was employed for bacteria and fungi count. The total coliform counts ranged from 2.32 to 3.15 log cfu/g; faecal coliform counts ranged from 1.58 to 2.58 log cfu/g; enterococci count ranged from 1.06 to 1.90 log cfu/g; and fungal count ranged from 2.57 to 3.73 log cfu/g. There was a significant difference in the mean faecal coliform and enterococci counts for all fruit samples. Tiger nut had the most bacteria, while watermelon was the most fungi contaminated fruit. Shigella spp. was the commonest bacteria contaminates of fresh-cut ready-to-eat fruits sold in Kumasi. Giardia lamblia was the most abundant parasite detected. Fresh-cut ready-to-eat fruits and vegetables sold in markets and bus terminals in the Kumasi Metropolis, Ghana, are contaminated with bacteria, parasites, and fungi. These findings emphasize the need for public awareness and proper hygiene practices to reduce the risk of food-borne illnesses associated with the consumption of fresh-cut fruits.

### 1. Introduction

Consumption of fruits and vegetables has increased as people recognize the importance of a healthy diet. Fruits and vegetables contain essential nutrients such as sugars and fibres which are beneficial to human health. They are also a trusted natural source of essential vitamins and minerals needed by the body. Studies have shown that regular consumption of fruits and vegetables can lead to a healthier lifestyle and reduce the risk of chronic diseases such as cancer and heart disease [1–3]. Fruits and vegetables are relatively low in calories, making them a good option for weight control. Thus, including fruits and vegetables in one's diet is highly recommended for a healthy lifestyle. In Ghana, there has been a significant increase in the demand for fruits and vegetables, with mango being the most commonly consumed fruit [4].

Despite the well-known health benefits of fruits, there is a growing concern over the potential risks associated with consuming fresh-cut ready-to-eat fruits. In recent years, outbreaks of human diseases associated with the consumption of contaminated fruits and vegetables have increased [5, 6]. Epidemiological studies have revealed that fruits and vegetables are home to a variety of medically significant pathogenic parasites and bacteria such as *Salmonella* spp., *E. coli* O157:H7, *Shigella* spp., and *Bacillus*  cereus, as well as parasites such as Cyclospora spp., Cryptosporidium spp., Toxocara spp., and Trichuris trichiura, and have previously been isolated from fruits and vegetables [7–9]. In both developed and developing countries, the prevalence of food-borne illness has been a major public health concern. In many developing countries, the outbreak of food-borne illnesses is often attributed to factors such as inadequate toilet facilities, lack of fresh and clean water supply, low socioeconomic development, increasing human population, poor sanitation, low standard of living, malnutrition, suboptimal public health practices, and weaker healthcare systems, which are ill-equipped to effectively manage and mitigate the impact of such infections [10-12]. Therefore, developing countries tend to experience more severe health consequences arising from food-borne infections compared to their developed counterparts [13]. Food-borne illnesses have a significant economic impact, causing major disruptions and contributing to slow development in sub-Saharan African countries. The high burden of such illnesses also hinders progress towards achieving health-related sustainable development goals.

The contamination of fruits and vegetables by parasites and bacteria is mainly linked to the use of untreated wastewater for irrigation, the application of animal manure, and the handling of fruits and vegetables with microbeinfected hands [14-16]. Fruits and vegetables are exposed to various microbial agents during harvesting, handling, and storage, making them susceptible to bacterial, viral, and parasitic infections. Some fruits and vegetables have a spongy exterior surface, which facilitates parasite attachment and survival, increasing the risk of contracting a foodborne infection after consuming them fresh and uncooked. Although street-food vending is an important source of employment and income generation for many people in most developing countries, the sale of ready-to-eat food items on the open streets poses a risk to food safety [17]. As such, it is imperative to address the causes of food-borne illnesses in developing countries by promoting healthy sanitation practices, enhancing food-safety protocols, improving access to clean water, and increasing awareness of the risks associated with consuming raw or undercooked fruits and vegetables. Such measures can significantly reduce the incidence of food-borne infections and their associated morbidity and mortality rates.

Typically, many Ghanaians who buy fruits in markets and on the roadside do not wash them before consumption. Most of them assume that these fruits are clean enough for consumption. The potential health risks associated with consuming contaminated fruits and vegetables make it crucial to analyse the quality of those sold in markets in Ghana. The authorities from the Kumasi metropolis often embark on public health education for food vendors. However, there is no evidence of its impact on the safety of ready-to-eat fruits sold in markets in the metropolis. This study therefore aimed to assess the bacterial, fungal, and parasitic contamination of fresh-cut ready-to-eat fruits and vegetables that require little or no preparation before consumption in some markets in the Kumasi Metropolis in Ghana, with the goal of providing data to inform the implementation of appropriate interventions to reduce the risk of food-borne illness associated with vegetable consumption.

#### 2. Methodology

2.1. Study Design and Collection. A cross-sectional study was conducted in Kumasi at the local markets and bus terminals. Kumasi, the Ashanti region of Ghana, lies between latitude 6.35°N to 6.40°N and longitude 1.30°W to 1.35°W, with an elevation of 250 m to 300 m above sea level. It is in the transitional forest zone with the average temperature range being between 21.5°C and 30.7°C. Kumasi has double rainfall seasons making it conducive for cultivation of different fruits and vegetables [18]. As the second largest city in Ghana, Kumasi is home to some of the largest markets and bus terminals in Ghana. Samples were collected from six different locations in Kumasi, namely, Tech Junction, Asafo market, Adum, Abinkvi market, Sofoline market, and Atonsu market, as shown in Figure 1. These locations represent some of the busiest markets and bus terminals in the central business area of Kumasi.

Fresh-cut fruits displayed for sale or served in retail outlets were purchased from local markets and retail outlets in bus terminals. For the purposes of this study, fresh-cut fruits refer to fruits that have been cut open and sliced into pieces but remain in the fresh state. Overall, fifty-four samples each of five different fruits (total 270) commonly sold on the streets and markets were sampled. For each location, nine samples of each fruit used in the study were collected. Fruits sampled were pineapple (Ananas comosus), pawpaw (Carica papaya), watermelon (Citrullus lanatus), sugarcane (Saccharum officinarum), and tiger nut (Cyperus esculentus). Pineapple, pawpaw, and sugarcane had been cut and packaged in light transparent polythene bags, while watermelons had been cut and displayed on silver pans by vendors. Tiger nuts were also packaged in light transparent polythene bags. The collected samples were put in sterile plastic bags, labelled, and transported on ice to the Department of Theoretical and Applied Biology's Microbiology Laboratory for bacteriological analysis within 24 hours after collection.

2.2. Isolation and Enumeration of Bacteria. Samples were analysed for the presence of total and faecal coliforms, Salmonella spp., Shigella spp., E. coli, and Enterococci using the standard methods published in the Bacteriological Analytical Manual (BAM) by the Food and Drug Administration, Washington DC, USA [19–21]; Andrews et al. [22]. For each sample, 10 g was pulsified in 90 ml of buffered peptone water, following which the rinsed portion was serially diluted to  $10^{-1}$ ,  $10^{-2}$ ,  $10^{-3}$ ,  $10^{-4}$ ,  $10^{-5}$ , and  $10^{-6}$  dilutions in buffered peptone water. Using the Pour Plate Technique, 1 ml of each dilution was added to different agar for the isolation and enumeration of different bacteria. Following a standard protocol as described in the BAM (2021), bacterial pathogens were identified based on their morphological, cultural, and biochemical characteristics.

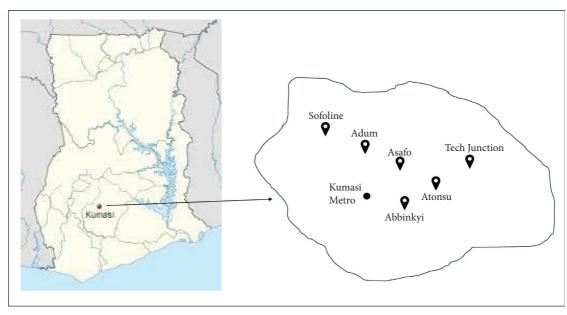


FIGURE 1: Map showing the study sites (source: town and country planning department, 2014).

The five-tube most probable number (MPN) technique was used to enumerate the total and faecal coliform load using MacConkey broth. The tubes positive for faecal coliforms were further analysed for the presence of *E. coli* using tryptophan broth inoculated with Kovacs reagent as performed previously. Eosinmethylene blue agar was further used to confirm the presence of *E. coli*. Slanetz and Bartley agar was used to isolate *Enterococci*, while Selenite Cystine broth base solution and Salmonella Shigella (SS) agar were used to isolate *Salmonella* and *Shigella*.

2.3. Isolation and Identification of Yeast and Moulds. The yeast and moulds were enumerated using the pour plate technique on yeast extract-peptone dextrose (YPD) agar. One millilitre (ml) of each of the  $10^{-1}$  to  $10^{-9}$  dilutions of test samples obtained from the pulsified samples was transferred into separate Petri dishes, and 20 ml of YPD agar was poured and gently swirled to mix. The plates were then incubated at 25°C for five to seven days. The colonies formed on the media were counted using a colony counter. The yeast and mould counts were expressed as colony forming units (CFU) per ml. Yeast and mould isolates were classified based on the standard cultural and morphological characteristics as described by the authors in reference [23]. The total yeast and mould counts were enumerated using the formula adopted by the authors in reference [23].

2.4. Isolation of Intestinal Parasites. Macroscopic examination was carried out on the samples to detect the presence of worms and larval forms of insects. Fifty grams of each sample were weighed and immersed in 150 mL of buffered peptone water contained in a sterile stomacher bag for a duration of one hour. The stomacher bag was shaken vigorously for two minutes, after which the contents were transferred into a new sterile stomacher bag. This process was repeated four times, and all resulting washes were collected and pooled into a beaker. Subsequently, a portion of the pooled wash was dispensed into tubes and centrifuged at 5,000 rpm for 5 minutes. Following this, the supernatant was carefully removed, and the sediments were resuspended in 1 ml of saline solution for parasitological examination. A drop of the resuspended sediment was placed on two clean, grease-free microscope slides. One of the slides was treated with a drop of iodine before microscopic examination. Each slide was subsequently examined under a light microscope to identify the presence of parasite forms. An additional slide was prepared for acid-fast staining using the modified acidfast (Ziehl–Neelsen) method as described previously [24].

2.5. Statistical Analysis. The data obtained were entered into Microsoft Excel and analysed using IBM SPSS statistics software version 20.0 (SPSS, 2010). Descriptive analysis was carried out on the various microbial count recorded. Afterwards, two-way analysis of variance (ANOVA) at 95% confidence interval was used to ascertain the statistical significance and association between microbial loads.

#### 3. Results and Discussion

In many developing countries, food is a major vehicle for the transmission of bacteria belonging to the Enterobacteriaceae family such as *E. coli*, *Salmonella* spp., and *Shigella* spp. [25–27]. In this study, we assessed the bacterial, fungal, and parasitic load of fresh-cut ready-to-eat pineapples, pawpaw, watermelon, sugarcane, and tiger nut sold in the major markets and streets in the Kumasi metropolis.

Microbial organisms were detected in fruits sold by vendors in various market streets within the Kumasi metropolis, suggesting substandard handling and processing of these freshcut fruits. The sampled fruits harboured different levels of total coliforms, faecal coliforms, enterococci, and fungi.

Fruit		Total mean microbial counts (log cfu/g)	
riult	Total coliform	Faecal coliform	Enterococci
Pineapple	2.64	2.00	1.17
Watermelon	2.82	1.79	1.14
Pawpaw	2.32	1.58	1.13
Sugarcane	2.33	1.68	1.06
Tiger nut	3.15	2.58	1.90

TABLE 1: Total geometric mean of bacterial load of some fresh-cut ready-to-eat fruits in the Kumasi Metropolis.

TABLE 2: Bacterial contaminates of fruits sold in Kumasi Metropolis, Ghana.

Fruit	Number examined	Number of sampl	es contaminated with each spec	cies of bacteria (%)
Fruit	Number examined	E. coli	Salmonella spp.	Shigella spp.
Tiger nut	54	16 (29.63)	17 (31.5)	18 (33.3)
Watermelon	54	17 (31.5)	16 (29.63)	13 (24.1)
Pineapple	54	15 (27.8)	10 (18.5)	14 (25.9)
Pawpaw	54	9 (16.7)	5 (9.3)	13 (24.1)
Sugarcane	54	10 (18.5)	5 (9.3)	10 (18.5)
Total (%)	270	67 [24.81%]	53 [19.62%]	68 [25.18%]

3.1. Total Coliform, Faecal Coliform, and Enterococci Counts in *Fruits*. The mean loads of total coliform, faecal coliform, and enterococci expressed as colony forming units per gram (cfu/g) were different between the different fruit samples as shown in Table 1.

Notably, the total coliform count exceeded the faecal coliform count, indicating that the primary source of contamination was soil rather than faecal matter. These findings align with similar investigations conducted in various regions of Ghana, as well as other developing nations [25, 28, 29]. The detection of *E. coli, Salmonella* spp., and *Shigella* spp. in the fruits is similar to previous studies conducted in Ghana by Nyarko et al. [30] and in other developing countries [31–33].

Tiger nut samples recorded the highest count of total and faecal coliforms (3.15 log cfu/g and 2.58 log cfu/g, respectively), while pawpaw samples had the least count of total and faecal coliforms (2.32 log cfu/g and 1.58 log cfu/g, respectively). For enterococci growth counts, tiger nut samples were the highest with 1.90 log cfu/g, while sugarcane (1.06 log cfu/g) had the least counts. Tiger nut samples consistently exhibited the highest bacterial counts across multiple locations, which is consistent with previous studies conducted by Ayeh-Kumi et al. in Accra, Ghana [34], and Nyarko et al. in Cape Coast, Ghana [30]. Ayeh-Kumi et al. identified five bacterial species in tiger nuts sold in Accra markets [34], while Nyarko et al. reported 16.2% of coliform contaminants of tiger nuts as *Enterococcus* spp. which serve as reliable evidence of faecal contamination of tiger nuts sold in Cape Coast, Ghana [30]. The highbacterial contamination found in tiger nuts can be ascribed to their distinct characteristics and handling procedures. It is worth noting that apart from tiger nuts, the epicarps of all fruits under study are peeled prior to consumption. In contrast, tiger nuts are consumed in its entirety, including the intact epicarp. In addition, the nonuniform nature of the epicarps on tiger nuts poses challenges in effectively cleaning them, unlike the uniform

TABLE 3: Total geometric mean of fungi load of some fresh-cut ready-to-eat fruits in the Kumasi Metropolis.

Fruit	Total mean fungi counts (log cfu/g) Fungi
Pineapple	3.11
Watermelon	3.73
Pawpaw	3.00
Sugarcane	2.57
Tiger nut	2.58

epicarps found on other fruits. Consequently, the surface of tiger nuts tends to retain dirt particles more easily than other fruits, thus increasing the likelihood of contamination. The comparatively high contamination levels observed in tiger nuts may also be attributed to their growth pattern within the soil, where the entire outer rind is fully submerged in the soil. Consequently, there exists a significant likelihood for the nuts to come into contact with soilborne nematodes and total coliform [35–37].

3.2. Distribution of E. coli, Salmonella spp., and Shigella spp. on Fruits and Vegetables. The results of this study showed that 188 (69.6%) fruit and vegetable samples were contaminated with either E. coli, Salmonella spp., and Shigella spp. Shigella spp. (25.18%) and E. coli (24.81%) were the commonest bacteria contaminates of the fruits and vegetables sampled. Salmonella spp. was the least contaminant of the three isolated bacteria (19.62%) (Table 2). There was no statistical significance between the different bacteria isolated from the fruit samples (p > 0.05). According to the Health Protection Agency in UK and the International Commission on Microbiological Specifications for Foods, consumable foods that are ready to eat must not contain Salmonella spp. and Shigella spp. as eating food infected with these pathogens could cause food-borne sickness. For *E. coli*,  $cfu/g > 10^2$  is unsatisfactory [38, 39]. The detection of these bacteria in the

Fruit/	Munchen					Number cc	Number contaminated with parasite	ith parasite				
Vegetable type	examined	Ascaris sp.	Hookworm	Trichuris trichiura	Strongyloides stercoralis	Enterobius vermicularis	Enterobius Entamoeba ermicularis coli	Entamoeba histolytica	Giardia lamblia	Isospora belli	Cryptosporidium parvum o	Cyclospora cayetanensis
Pineapple	54	4	4	6	14	0	3	6	6	0	4	1
Watermelon	54	26	23	13	33	12	18	28	32	12	33	18
Pawpaw	54	0	0	0	9	0	0	1	19	0	2	0
Sugarcane	54	18	15	10	6	0	0	2	15	0	0	0
Tiger nut	54	35	23	20	27	6	27	28	33	8	29	19
Total	270	83 [30.74%]	83 [30.74%] 65 [24.07%] 49 [18.14%]	49 [18.14%]	89 [32.96%]	21 [7.00%]	21 [7.00%] 48 [17.00%] 68 [25.18%]	68 [25.18%]	108 [40%]	20 [7.40%]	68 [25.18%]	38 [14.07%]

TABLE 4: Intestinal parasite contaminates of fruits sold in Kumasi Metropolis, Ghana.

fruits is therefore indicative of inadequate preparation and handling procedures.

3.3. Fungal Contamination of Fruits and Vegetables. Watermelon samples exhibited the highest fungal counts among all locations, with a mean count of 3.73 log cfu/g, whereas sugarcane and tiger nuts displayed the lowest fungal counts, as shown in Table 3.

Analysis of the mean counts varied significantly in all fruit counts except for sugarcane (p = 0.375). For pineapple, p = 0.057; watermelon, p = 0.001; pawpaw, p = 0.011; and tiger nut, p = 0.001. For the test of homogeneity of variance, there was significance in pineapple, watermelon, pawpaw, sugarcane, and tiger nut mean counts (p = 0.001).

The presence of fungal contamination can be attributed to factors such as contaminated soil, unclean utensils (e.g., knives, cutting boards, washing bowls, trays, or pans), improper handling practices or procedures, substandard personal hygiene of vendors, and air-borne fungal particles. A similar investigation identified *Aspergillus* spp. contamination in processed watermelon fruit sold in the Koforidua market in Ghana [40]. The susceptibility of fresh-cut watermelon to contamination can be attributed to its low acidity and specific growing conditions [41, 42].

3.4. Intestinal Parasite Contamination of Fruits and Vegetables. Intestinal parasites were detected in most of the fresh-cut fruit samples collected from the study locations, and the number of counts varied among the samples analysed as shown in Table 4. A total of five intestinal helminths were detected, including Ascaris sp., hookworm, Trichuris trichiura, Strongyloides stercoralis, and Enterobius vermicularis. For intestinal protozoa, a total of six were detected, including Entamoeba coli, Entamoeba histolytica, Giardia lamblia, Isospora belli, Cryptosporidium parvum oocysts, and Cyclospora cayetanensis. Giardia lambia (40%) was the most prevalent intestinal parasite contaminating the fruits, with Ascaris lumbricoides and Strongyloides stercoralis also recording high prevalence (30.74% and 32.96%, respectively). Similar studies in Cameroon detected parasite contamination on fresh pawpaw, watermelon, and pineapple with E. histolytica/E. dispar (29.4%) being the most frequent, followed by Entamoeba coli (11.8%) and Giardia lamblia (6.1%) [43]. Most studies of parasite contamination of fruits and vegetables in developed countries reveal high contamination with common intestinal parasites of medical importance to humans [16, 34, 44].

Fresh-cut ready-to-eat fruits are susceptible to contamination by both pathogenic and nonpathogenic microorganisms because of how they are prepared and handled before and during sale. Factors such as handling with contaminated hands, direct exposure to soil, and the use of contaminated water for irrigation and fruit washing contribute to the proliferation of a diverse array of bacteria, fungi, and parasites that contaminate these fruits [45–47]. Ayeh-Kumi et al. also identified the increase of urbanization as an additional contributing factor to the contamination of fruits available in urban Ghanaian communities. According to them, urbanization has exerted substantial strain on essential resources such as potable water, which is necessary for the thorough cleansing of fruits and vegetables. Consequently, vendors resort to utilizing bowls of water instead of running water for washing these produce items [34]. Edusei et al. also made a noteworthy observation regarding vendors who employ repeated use of water for washing fruits without replacing it, potentially leading to contamination. This practice raises concerns regarding food safety. In addition, they reported that a majority of these vendors lacked formal training in food processing and personal hygiene, specifically pertaining to fresh-cut fruits [48].

The microbial contaminants isolated from the fruits could be responsible for many cases of food-borne illness in Ghana and many developing countries. While they might not cause large mortality, these illnesses lead to loss of productivity as well as substantial costs in hospitalization [49–51].

#### 4. Conclusions and Recommendations

Fruit samples analysed exhibited varying degrees of microbial contamination with medically important pathogens. Total coliforms, faecal coliforms, and enterococci were detected in the fresh-cut ready-to-eat fruits. Among the fruits studied, tiger nut samples demonstrated higher bacterial and parasite loads. Moreover, fruit samples were contaminated with fungi, with watermelon and pineapple samples exhibiting higher fungi load. Notably, fruits with higher pH levels and lower water content demonstrated lower fungal loads.

The sale and consumption of fresh-cut ready-to-eat fruits, such as pineapples, watermelon, pawpaw, sugarcane, and tiger nuts, have become popular among Ghanaians. Therefore, the findings of this study hold significant implications for public health and emphasize the need for appropriate interventions to prevent the transmission of bacterial pathogens and fungi associated with the consumption of contaminated fresh-cut fruits. To ensure the hygiene of freshly cut fruits, it is generally recommended that they undergo thorough washing and rinsing using running potable water before sale and consumption. Workshops on public health and the need for personal hygiene should be organised for fresh-cut ready-to-eat fruit vendors by the Kumasi Metropolitan Assembly.

#### **Data Availability**

The data that support the findings of the study are available upon request.

#### **Conflicts of Interest**

The authors declare that they have no conflicts of interest.

#### **Authors' Contributions**

JAL and KB conceived and designed the study. GE and VS took and analysed samples. KB and JAL drafted the manuscript. All authors reviewed and approved the final manuscript for submission.

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