

Original Research Article

<https://doi.org/10.20546/ijcmas.2023.1204.001>

## Microbiomes of Selected Commercial Fufu Grinding Machines, Mortars and Pestles Used at Homes in Ayeduase, A Suburb of Kumasi

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### ABSTRACT

Food safety, which focuses on disease-causing bacteria and their toxins that may contaminate the food, is the essential component of food microbiology. This is why it is crucial to understand the potential contribution of microbes linked to particular diets. In this article we compared microbiomes (bacteria and fungi) of commercial fufu grinding machines, mortars and pestles used at selected homes in Ayeduase, a suburb of Kumasi. Samples were taken from three distinct locations for fufu grinding machines and three different places for traditional procedure using mortar and pestle. Sample collection was done at each site and home twice a day, before work began and after the day's work. Samples collected from grinding sites were coded G1S1, G1S2, G2S1, G2S2, G3S1 and G3S2, while samples collected from homes were codes T1S1, T1S2, T2S1, T2S2, T3S1 and T3S2. Morphological, biochemical characterization and microscopic identification were done for all the isolates using standard bacteriological methods. Bacteria counts of samples from fufu grinding machines ranged between  $7.5 \times 10^{11}$  and  $2.73 \times 10^{12}$  CFU/ml, while those obtained from mortars and pestles ranged between  $2.5 \times 10^{11}$  and  $1.96 \times 10^{12}$  CFU/ml. This result showed there was no significant difference between the samples obtained from grinding machines and samples obtained from mortars and pestles statistically ( $p > 0.05$ ). All twenty-nine pure culture isolates for bacteria sub-culturing were Gram positive from Gram staining. The dominant isolates were *Staphylococcus* sp. (65.5%), *Diplococcus* sp. (13.79%), *Streptococcus* sp. (10.34%) and *Bacillus* (10.34%). Fungal morphology and identification of samples were also done based on standard identification keys. The dominant fungi genera identified were *Trichophyton*, *Aspergillus*, *Fusarium*, *Blastomyces*, *Cladosporium* and *Penicillium*. This study also concluded that bacteria and fungi genera associated with commercial fufu grinding machines and mortars and pestles are *Staphylococcus*, *Diplococcus*, *Streptococcus*, *Bacillus*, *Trichophyton*, *Aspergillus*, *Fusarium*, *Blastomyces*, *Cladosporium* and *Penicillium* and that grinding machines have higher numbers of bacteria and fungi as compared to mortars and pestles.

#### Keywords

Food safety,  
Microbiomes, fufu  
grinding machines,  
mortar and pestle

#### Article Info

**Received:**

05 March 2023

**Accepted:**

01 April 2023

**Available Online:**

10 April 2023

## **Introduction**

In West and Central Africa, as well as other regions of the world including the Caribbean and many countries with a sizable population of West African heritage, fufu is a popular dish. Foods that resemble dough are called "fufu" in West African cuisine. Along with Ghana, other countries that use it include the Central African Republic, Sierra Leone, Cote D'Ivoire, Togo, Cameroon, Nigeria, Guinea, Angola, Liberia, the Democratic Republic of the Congo, Benin, Republic of Congo, and Gabon (Tuomainen, 2009). Traditionally, it is prepared in Ghana, Ivory Coast, Liberia, and Cuba by crushing equal parts of cooked cassava with green plantain or cocoyam separately, or by combining flour formed from cassava, plantain, or cocoyam with water and stirring it over a stove.

It is a starchy staple with deep roots in Ghanaian history and mainly made from pulsating cooked cassava or yam, mostly with added cooked unripe plantain or cocoyam in a mortar with a pestle (Feglo and Sakyi, 2012).

The techniques and methods required to prepare fufu vary greatly, depending on the location. Fufu is often combined using a wooden pestle and a locally crafted wooden mortar. The mixture is rotated by hand in between pestle blows as water is gently added until it turns into slurry, mushy, and sticky (McCann, 2009). After that, the mixture is split into a slab and dished. Fufu is frequently served in Ghana with lukewarm or hot liquid soups such as groundnut soup, light soup and palm nut soup. This traditional process requires a lot of energy and it is also time consuming. It is generally considered unhygienic due to the use of the hand and water to drive the crushed foodstuff repeatedly to get a smooth consistency (Gbasouzor and Mbunwe, 2016). Accordingly, efforts have been made to abandon the conventional technique, which requires for the use of a mortar and pestle (Addo *et al.*, 2020). Recently, advancement in technology has led to new and innovative methods of preparing fufu such as the invention of the fufu grinding machine.

The fufu grinding machine is a motorized machine developed to help grind fufu easily and end the tedious means of preparing the fufu meal by pounding with a mortar and pestle (Ogwu *et al.*, 2001). The fufu grinding machine has the ability to grind boiled cassava, cocoyam, plantain, yam or a mixture of two or more, to meet the individual's taste within few minutes depending on the quantity to be milled (van Rheenen *et al.*, 2012). The machine is touted as being able to grind fufu with less labor.

In spite of the less labor provided by the recent advancement in technology for fufu preparation, it still suffers from the challenge of lack of hygienic practices by its operators. This is due to the possibility of fostering the development of harmful and spoilage microbes in cassava and its products, similar to other food components (bacteria and fungi). As such, one is not sure whether the machine traps microorganisms which may end up contaminating the fufu especially if the machine is not cleaned in between pounding. The risk of introducing microbiological pollution during processing and shipping is also very high because these devices are placed in the marketplace and the community. Furthermore, the possibility of commercial fufu operators not cleaning the machine routinely which might cause food particles to stay between the grooves and become a source of microbial contamination. This might put consumers' health in danger (Addo *et al.*, 2020).

Inspired by the concept of food security and food microbiology, which concentrates on the disease-causing microorganisms and their pollutants which may pollute the foods such as fufu. It has therefore become crucial to understand the vital implication of microorganisms associated with certain foods, especially ready to eat foods to ensure public safety. The objective of this research is to identify and differentiate the various types and numbers of bacteria and fungi associated with selected commercial fufu grinding machines to fufu apparatus used at selected homes in Ayeduase, Kumasi.

## **Materials and Methods**

An experimental study to determine and compare the various types and numbers of bacteria and fungi associated with selected commercial fufu grinding machines to fufu apparatus used at selected homes in Ayeduase, Kumasi. The study was conducted in Ashanti region's Ayeduase, in the Oforikrom submetro of Kumasi Metropolitan Assembly. Ayeduase is situated nearby Kotei and west of Boadi. It has coordinates of 6° 40' 30" N latitude and 1° 33' 34" W longitude. The laboratory analysis was conducted in the Microbiology laboratory of KNUST's Department of Theoretical and Applied Biology.

### **Sample Collection**

Here, three (3) grinding shops were conveniently selected at Ayeduase and sampled. Duplicate samples were taken, in the morning before operating the machine and in the evening after the day's work from the commercial grinding machines from each of the three grinding shops. Sterile but moistened cotton swabs were used to swab the inside of the hooper, milling compartment and the part that extrudes the grinded fufu. The samples collected were labelled as G1S1, G1S2, G2S1, G2S2, G3S1, and G3S2. In addition, three homes that traditionally prepare fufu using a mortar and pestle were conveniently chosen and tasted. Duplicate samples were taken from the mortar and pestle before and after fufu had been pounded.

To clean the surfaces of the mortar, its crevices, and the bristles of the pestle, sterile cotton tip swabs were wet with 10ml of peptone water, just like when using a grinding machine. T1S1, T1S2, T2S1, T2S2, T3S1, and T3S2 were the labels assigned to the samples after collection.

All of the samples were then sealed in zip-top bags and kept chilled in a thermos flask. The samples were subsequently delivered to the microbiology lab at the KNUST Department of Theoretical and Applied Biology for further analysis.

## **Laboratory Procedure**

In this section, laboratory and experimental procedures are explained.

### **Microbiological Analysis**

To begin with the experimental analysis, serial dilutions of the samples were created. A pulsifier was used to homogenize the samples taken with the cotton tip swabs in zip lock bags. 1.0ml of each sample was taken using a micropipette and a sterilized 1.0ml pipette tip. This was then added to a 9.0 ml sterile distilled water (diluent) in a sterile test tube. Dilution of each of millimeter of the sample was in 10 folds. A new sterile pipette tip was used to take 1.0ml of the 10<sup>-1</sup> dilution and pipette it into a sterile tube labelled 10<sup>-2</sup>. This was repeated serially until the highest dilution factor of 10<sup>-10</sup> was reached.

Furthermore, bacterial culturing and identification was done. Here, a volume of 1ml of diluted serial factor of distilled water from 10<sup>-1</sup> to 10<sup>-4</sup> for each sample were pipetted into 4 petri sterile petri dishes containing plate count agar and shaken gently. It was left undisturbed on a flat surface and allowed to solidify. It was then incubated for 18-24 hours at 37° C. A colony counter was used to tally the number of produced colonies. Morphological identification of the colonies was done using the colony description according to shape, size, margin, colour. The various forms of colonies for each sample were used for sub-culturing for further isolation and testing. Using a sterile loop, the various colony types were removed from the plate count agar onto plastic petri dishes containing nutrient agar and streaked gently. These were incubated at 37° C for 18-24 hours.

Another major contribution to our analysis is also the identification and culturing of fungi on our samples. Here, a volume of 1ml of diluted serial factor of distilled water from 10<sup>-1</sup> to 10<sup>-4</sup> for each sample were pipetted into 4 petri sterile petri dishes containing Potato Dextrose Agar and shaken gently. It was left undisturbed on a flat surface and allowed to solidify. It was then incubated for 5-7 days at

30°C – 35°C. A colony counter was used to tally the number of produced colonies. The growth characteristics of the fungi colonies on the growth media will give indication about the fungi. This is because different fungi will produce differences in colonies on the growth media. These characteristics include texture and surface color.

### **Bacterial Isolate Identification**

After the bacterial isolates had been divided into groups for examination, the colonies were counted, and different colonies were selected at random to perform several physiological and biochemical tests.

The catalase test, indole test, methyl red test and citrate test were some of the biochemical tests carried out in this study. To distinguish between gram-positive and gram-negative bacteria, gram straining was also used.

### **Data Management and Analysis**

Using Microsoft Word and Excel, the laboratory's data were entered. To determine the mean and standard deviation of the various data sets, descriptive statistics were utilized. ANOVA was used to compare the variances between the means of the data sets for various variables.

### **Some Experimental Precautions**

Petri dishes were inverted when inoculating to prevent condensation which would disrupt the isolation technique (that is; the condensed moisture formed from the perspiration of the bacteria will alter their growth pattern, making it less visible). All glass wares used for the experiment were sterilized to avoid contamination.

The autoclaved growth media flamed tip of the test tubes and loop were allowed to cool after sterilization to prevent the killing of microorganisms. Care was taken not to scrape the agar in addition to the cells to avoid false-positive results.

## **Results and Discussion**

### **Determination of Total Bacteria Load in fufu grinding machine samples and mortars and pestles samples**

The mean Total Viable Count (TVC) in the grinding machines from the 'before usage of the machine' was  $1.39 \times 10^{12}$  CFU/ml and that of the 'after usage of the machine' was  $1.63 \times 10^{12}$  CFU/ml in all the grinding sites. Statistically, the differences in the mean count from the various receptacles were not significant ( $p=0.725$ ).

The mean Total Viable Count (TVC) on the mortars and pestles before pounding was  $8.67 \times 10^{11}$  CFU/ml and that of after pounding was  $6.13 \times 10^{11}$  CFU/ml. Statistically, the differences in the mean count from the various receptacles were not significant ( $p=0.687$ ). The mean total viable count of the apparatus's bacterial load is displayed in Table 1.1

### **Isolates and Biochemical Tests**

From all the samples taken using mortar and pestles and grinding machines, 29 pure cultures were isolated. On the basis of shape, size, colour, margin, elevation, and opacity, various colonies were segregated. All the 29 isolates were Gram positive from the Gram staining reaction.

The dominant bacteria isolated from all the samples are shown in Table 1.5 below. These bacteria were recognised as *Staphylococcus* species, *Streptococcus* species, *Diplococcus* species, and *Bacillus* species. *Staphylococcus* sp. was the most prevalent isolate in the sample ( $n = 19$ , 65.5%), followed by *Diplococcus* sp. ( $n=4$ , 13.79%), *Streptococcus* sp. ( $n=3$ , 10.34%) and *Bacillus* sp. ( $n=3$ , 10.34%).

As indicated below in Table 1.2, when all the isolates were subjected to biochemical tests, they all reacted positive for Gram staining. Out of the 4 isolates, three (3) were cocci shaped and one (1) was rod shaped. All the isolates tested negative for both Indole and Citrate test. Three (3) tested negative for

Methyl red test and two (2) tested positive for Catalase test. Probable candidate bacteria were *Staphylococcus sp.*, *Streptococcus sp.*, *Diplococcus sp.* and *Bacillus sp.*

### **Identification of Fungal Isolates in Apparatus**

All twelve samples cultured showed the presence of fungal growth on Potato Dextrose Agar (PDA) plates. Six (6) different fungal species were morphologically identified in the various PDA plates based on surface colour and texture. These fungal isolates were identified to be *Fusarium*, *Trichophyton*, *Penicillium*, *Blastomyces*, *Cladosporium* and *Aspergillus*.

As indicated in Table 3 below, *Trichophyton* had the highest number and frequency in both grinding machines and mortars and pestles. *Penicillium* had the least number and frequency in all grinding machines and *Cladosporium* had the least number and frequency in all mortars and pestles.

### **Bacteria load in commercial fufu grinding machine samples and mortar and pestle samples**

The test result in Section 3 from Table 1 showed that the commercial fufu grinding machines' mean count of bacteria load ( $1.51 \times 10^{12}$ ) were relatively higher than the samples taken from mortars and pestles ( $7.4 \times 10^{11}$ ). This study agrees with a survey conducted by Addo *et al.*, (2020) and Amreeta *et al.*, (2015), and compares it to numerous other investigations. Amreeta *et al.*, (2015) based their argument on the fact that the grinding machine has numerous crevices (auger, shaft, pounding compartment, hopper and discharge outlet) which provide a favorable environment for the development of bacteria. The area where these grinding machines are situated could potentially serve as a source of contamination due to the presence of filthy floors, gutters, busy areas like marketplaces, and a nearby industrial facility. Environmental conditions such as pH, temperature, moisture and nutrient contents on the surfaces of the

crevices could harbor bacteria growth. Though the grinding machines had relatively higher bacterial load count as compared to the mortars and pestles, the difference was statistically insignificant. This counteract the hypothesis that fufu processed with grinding machines are more unhygienic as compared to mortars and pestles used in fufu processing. The higher levels of bacteria count are also relative and may be that the homes from which samples were taken from did not observe proper hygienic protocols which led to the higher counts.

The test also showed that, the mean bacteria count “before usage” of the machines and mortars and pestles were relatively higher than the “after usage” samples. This result can mean the cleaning methods used by both machine and traditional fufu handlers are not effective against the bacteria, though it is able to clear the debris of fufu from the surfaces of the grooves of the machines and the crevices of the mortar and pestles.

All the 29 isolates were Gram positive from the Gram staining reaction among which the dominant bacteria isolated were *Staphylococcus sp.* followed by *Diplococcus sp.*, followed by *Streptococcus sp.* and *Bacillus sp.* The presence of *Staphylococcus sp.* may be as a result of either of following sources of contamination: mixing and molding in the machine from skin (body contact with food), mouth (spit from talking) or nose (sneezing) of the machine handler or the pounder. It could also be introduced as a result of sweats from the machine operator or the pounder with the traditional processing fufu.

*Staphylococcus* is among the microorganisms that can cause food poisoning when consumed because of the toxins it produces. According to Mbaeyi *et al.*, (2010), environmental pollution and air pollution are additional potential sources of *Staphylococcus* contamination in fufu. *Diplococcus* and *Streptococcus* which mostly are part of the common flora of the microbial flora of both people and animals can cause acute infections, although some are harmless.

**Table.1** Mean Total Viable Count of Bacterial Load

Apparatus	Average Count (CFU/ml)	Range (CFU/ml)	p-value
Grinding Machines (Before Usage)	1.39X10 <sup>12</sup>	1.10X10 <sup>12</sup> -1.95X10 <sup>12</sup>	0.725
Grinding Machines (After Usage)	1.63X10 <sup>11</sup>	7.5X10 <sup>11</sup> – 2.73X10 <sup>12</sup>	
Mortars And Pestles (Before Pounding)	8.67X10 <sup>11</sup>	2.5X10 <sup>11</sup> – 1.96X10 <sup>12</sup>	0.687
Mortars And Pestles (After Pounding)	6.13X10 <sup>11</sup>	2.8X10 <sup>11</sup> – 9.8X10 <sup>11</sup>	

**Table.2** Biochemical and Morphology Characteristics of Bacterial Isolates

Gram reaction/ shape	+ (Cocci)	+ (Cocci)	+ (Cocci)	+ (Rod)
Test characteristics of Isolates	A	B	C	D
Morphological characteristics	Circular, white with convex and smooth colonies.	Irregular, creamy-white with umbonate and smooth colonies.	Circular, creamy-white with convex and smooth colonies.	Rhizoid, creamy-white with flat and smooth colonies
Catalase test	+	-	+	-
Indole test	-	-	-	-
Methyl Red test	-	-/+ (varied)	-	-
Citrate Test	-	-	-	-
Probable identity	<i>Staphylococcus sp.</i>	<i>Streptococcus sp.</i>	<i>Diplococcus sp.</i>	<i>Bacillus sp.</i>

A-Isolate A    B- Isolate B    C- Isolate C    D- Isolate D    (+) – Positive reaction    (-) – Negative reaction

**Table.3** Numbers and Frequency (%) of fungal isolates for the apparatus in general

Fungal Isolates	Number of Visible Colonies (All Grinding Machines)	Frequency (%)	Number of Visible Colonies (All Mortars and Pestles)	Frequency (%)
<i>Trichophyton</i>	1481	81.87	1014	59.37
<i>Aspergillus</i>	182	10.06	408	23.89
<i>Fusarium</i>	132	7.29	249	14.58
<i>Blastomyces</i>	9	0.50	29	1.70
<i>Cladosporium</i>	5	0.28	1	0.06
<i>Penicillium</i>	0	0	7	0.41
<b>TOTAL</b>	<b>1809</b>	<b>100</b>	<b>1708</b>	<b>100</b>

The presence of these in the grinding machines and mortars and pestles indicate contamination of the fufu products with humans or animals and therefore the need for hygienic protocols to be intensified. The presence of *Bacillus* which is mostly an inhabitant of the soil and raw plant foods. The presence of *Bacillus* in the grinding machines and mortars and

pestles samples can be as a result of contamination from the floor and raw materials of food substances Odom *et al.*, (2012).

**Fungal load in grinding machines samples and mortars and pestles samples**

The result of the total plate counts visible fungi

colonies showed relatively higher numbers of fungi genera in the grinding machines (1809) as compared to the fungi genera in mortars and pestles (1708). *Aspergillus*, *Fusarium*, *Trichophyton*, *Penicillium*, *Blastomyces* and *Cladosporium* were the dominant fungi genera identified from the plates in different frequencies. The high fungal numbers in the grinding machines and mortars and pestles signifies that, the grooves of the machine and crevices of the mortars and pestles over time creates a conducive and friendly niche for fungi to thrive. This might be explained by the substantial amount of fermented sugar in fufu constituents and the high moisture content from the fufu constituent like cassava (Ubuala, 2007). Also, the high numbers of fungi can be due to the ineffective cleaning of the machines and mortars and pestles by the handlers. Most machine operators clean their machines with just lukewarm water after the day's work and rinse with water before they begin work the next day. This allows fufu debris to stick and pile up in the grooves of the machine and thereby enhancing fungi growth. The water used for driving fufu during pounding and bringing the fufu to a desired texture when grinding can also be a source of contamination to the processed fufu. The storage condition of mortars and pestles and grinding machines can also create a conducive environment for fungi growth when there is moisture. These fungi genera found are most of the time associated with food poisoning because of the mycotoxins they produce. Some which have raised health concerns and are considered contaminants when found in food are *Aspergillus*, *Fusarium*, *Penicillium*. When optimum growth conditions are made available for these fungi genera, they produce mycotoxins which are harmful to the health of the consumers. The presence of these fungi genera (*Aspergillus*, *Fusarium*, *Penicillium*) may be as a result of contamination of the processed fufu with raw plant material like cassava roots, stem and leaves which are normal microflora of these fungi (Zhang *et al.*, 2021). Environmental conditions such as dirty floors, dust in air which harbor fungi genus like *Trichophyton* can also serve as source of contamination. The presence of *Trichophyton* which has the highest frequency in both grinding machines

and mortars and pestles indicates the processed fufu is contaminated and will therefore call for proper hygienic protocols to be put in place.

These high numbers of the fungi genera may be due to unhygienic storage conditions of the grinding machines and the mortars and pestles which support growth of these fungi. Again, the higher numbers of the fungi genera may be as a result of improper cleaning methods of grinding machines and mortars and pestles which may not be effective in getting rid of the fungi present. A range of soluble carbohydrates, including glucose, xylose, sucrose, and fructose, are easily absorbed and metabolized by fungi. Fufu with all its constituents having these soluble sugars is easily metabolized by fungi. The high number of the different genera of fungi could therefore be attributed to the conducive environment (sugars and moisture content) provided in the grooves of the machines, crevices of the mortar and brush of the pestle. The production of toxins by these fungi therefore calls for proper cleaning of the grinding machines and mortars and pestles.

The study showed that both grinding machines and mortars and pestles had high numbers of bacteria and fungi with the difference not been significant. The bacteria genera isolated were *Staphylococcus sp.*, *Streptococcus sp.*, *Diplococcus sp.* and *Bacillus sp.* The fungi genera also identified were *Aspergillus sp.*, *Fusarium sp.*, *Trichophyton sp.*, *Penicillium sp.* and *Cladosporium sp.* The high numbers of both bacteria and fungi in the grinding machines and mortars and pestles signified that, strict hygienic protocols should be observed by the machine operators and fufu consumers at the grinding shops and at home respectively to prevent contamination of the finished product. Proper hygienic measures like routine cleaning of machines, conducive storage of both machines and mortars and pestles, cleaning with hot water and disinfectants and also frequent sanitizing of hands and surfaces by operators would help reduce the level of contamination. Potable water should be used to drive along grinding and pounding fufu and should be changed frequently, if possible, from client to client at the grinding shops.

Additionally, the use of conventional bacteriological tests (culture dependent methods) which are less sensitive and less specific in identifying and characterizing microorganisms limited our study to only focus on bacteria and fungi leaving out the other pathogens associated with commercial fufu grinding machines and mortars and pestles.

In the future, we hope to use high through put techniques like molecular based tests which take into account sensitivity and specificity of pathogens in a short time and not just the viability as compared to conventional bacteriological tests in further studies. We also hope to include the identification of viruses and other microorganisms in our study to give a better overview of the microbiome of commercial fufu grinding machines and mortars and pestles.

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### How to cite this article:

Aboagye Hagar Serwaa, Achiaa Akoto Abigail, Amoateng Nana Akwasi and Adofo Daniel Asante. 2023. Microbiomes of Selected Commercial Fufu Grinding Machines, Mortars and Pestles Used at Homes in Ayeduase, A Suburb of Kumasi. *Int.J.Curr.Microbiol.App.Sci*. 12(04): 1-8.

doi: <https://doi.org/10.20546/ijcmas.2023.1204.001>