Evaluation of microbial quality of *Fufu* samples prepared using conventional and mechanical methodsin Kumasi metropolis

Abstract

Aim: The studyaimed atevaluating themicrobial quality of conventionally (CV) and mechanically (MC) prepared*fufu* samples in Kumasi Metropolis in Ghana.

Study design:The study used 2 x 5 x 2 experimental design constituting two *fufu* preparation methods (CV and MC), five different vending locations, and two sample types (*fufu* samples and water samples) from each vendor.

Materials and Methods:A total of ten (10)*fufu* and water samples each were conveniently collected and analysed using standard microbiological procedures.

Place and Duration of study: The study was carried out inthe Kumasi Metropolis of Ghana, inMarch 2022

Results: The total aerobic count in theconventional and mechanical prepared samples ranged from 5. 2×10^{7}cfu/g to 4.5×10^{8}cfu/g and 5. 4×10^{7}cfu/g to 1.35×10^{8}cfu/g respectively. The total aerobiccount inthe water samples ranged between 9.7×10^{6}cfu/ml and 1.35×10^{9}cfu/mlin(CV) and 1.53×10^{6}cfu/ml – 6.3×10^{8}cfu/mlin(MC). Both the *fufu* and water samples from the vendors had unsatisfactory levels oftotal and faecal coliform count, *Staphylococcus aureus* count, and yeast count irrespective of the processing method (CVandMC).

Conclusion: The *fufu* processing methods did not influence its microbial qualities positively. Hence Good Hygienic Practices are essential to ensure itsmicrobiological safety.

Keywords: microbial quality, *fufu*, Kumasi , cassava, food safety

Introduction

Fufu is a staple dish in western and central Africa that is thought to have generated from Akan people in Ghana[1].The majority of Ghanaians eat *fufu*" as a staple food especially the Akans. *Fufu* is typically processed in Ghana using two primary techniques, mechanical and convectional. *Fufu* is traditionally made by boiling unripe mature plantains, cassava, cocoyams or yamsuntil they are tender[2][3].After thetubers or plantains are cooked, they are crushed with a pestle and mortar, turning them over and over with wet hands until the mixture forms a dough [2].Ordinarily, one person stands and mechanically uses the pestle to pound the cooked ingredients in the mortar in rhythmic strokes, while the other person turns the ingredients in the mortar, with wet hands and intermittently adds water to form the "*fufu*" dough[4][1]. This is a time-consuming and hard operation, particularly if it needs to be prepared commercially or in large quantities. This resulted in the creation of the automated *fufu* processing method (using *fufu* machine), which requires less labour than the conventional approach[5].The components of the *fufu* machine include the electric motor,

shaft, trough, pulleys, propellers (yam beater), and a frame with vents to allow for proper cooling of the machine while it is in use.The *fufu*milling machine receives the cooked roots or tubers through an automated process. The *fufu* is subsequently mechanically processed by the mill and extruded as a dough. The extruded dough is briefly pounded and formed into balls for eating [2]. The prepared *fufu* is typically served with the soup of choice and the safety and quality of *fufu* is largely dependent on the hygienic practices of the producers. It is particularly perishable because it is often offered in the wet form (moisture content of roughly 50%)[6]. Studies on the microbiological quality of different street foods have been conducted in Ghana. The use of hands, utensils, and the environment or the water used to moisten hands when processing *fufu* can be a source of contamination for '*fufu*'[7]. These could inevitably lead to modifications in the food product's texture, taste, look, and fragrance, as well as a decline in its acceptability and safety[6].Mechanized *fufu* processing has become common in Ghana's cities, and most people choose to use it instead of the more antiquated pestle and mortar approach [1][8].A comparativeapproachto evaluate whether the mechanized processing of *fufu* will improve microbial load is therefore very essential. This study therefore aimed atthe evaluationof the microbial quality of *Fufu* samples prepared from conventional and mechanical methods in Kumasi metropolis.

Materials and Methods

Study Area

The study was carried out in the Kumasi Metropolis (Kejetia) of the Ashanti region of Ghana, West Africa

Sampling and Preparation

Ten (10) samplesof *fufu* and water used during processing were obtained from ten different *fufu* sellers (5 conventional and 5 mechanical) within the Kumasi Metropolis (Kejetia) of the Ashanti region.The *fufu* samples were collected into sterile Zip-lock bags using a sterile spatula which was sterilized with 70% ethanol prior to sampling. The water samples were collected into sterile sampling bottles, sealed and labelled accordingly. The *fufu* and water samples were transported with ice to the Microbial Biotechnology laboratory of the Department of Biochemistry, KNUST.

Microbial Analysis

Serial dilution

For all the samples, 5 grams were added to 45ml sterile peptone water and homogenized in a stomacher (Lad Blender, Model 4001, Seward Medical, England), for 30 s at normal speed. One millilitre aliquot of each dilution, prepared using suitable ten-fold dilutions, was directly inoculated into sterile Petri dish containing Mannitol Salt Agar, MacConkey agar, Plate Count agar and Potato Dextrose Agar and spread.

Enumeration of Total Aerobic Count (TAC), Total Coliform Count (TCC), *Staphylococcus aureus*, Yeast and Mould Count

Aerobic bacteria were enumerated by the spread plate method on Plate count agar (PCA)(Oxoid CM325; Oxoid Ltd., Basingstoke, Hampshire, UK). The plates were incubated

at 37 ºC for 48 hrs.Total coliforms and *E. coli* were determined using the spread plate technique on MacConkey agar. The plates were incubated at 37 ºC for 48 hrs. The appearance and color of coliforms was used to differentiate lactose fermenters from non lactose fermentors. The red colonies, which are lactose fermenters, were counted as suspected *Escherichia coli*were enumeratedby looking for colonies with a smooth texture. *Staphylococcus aureus* was determined by spread plate method on Mannitol Salt Agar (Oxoid Ltd, Hampshire, England). The plates wereincubated at 37 ºC for 24 hours. The number of yellow colonies wereenumerated as *Staphylococcus aureus*. Yeasts and moulds were enumerated by the spread plate technique on a fresh potato dextrose agar plate. The plates were incubated at 25 °C for a week.

Characterization and Identification of Bacteria Isolates from *Fufu*

The bacteria were isolated and identified using standard microbiological methods such as the catalase test,potassium hydroxide test, and Gram stain.

Statistical Analysis

The mean and standard deviation of the microbial count were subjected to analysis using SPSS version 23 and Microsoft Excel.

Results

Microbial Quality and Safety of *Fufu* Samples

Total Aerobic Bacteria Contamination of *Fufu* Samples

The amount of total aerobic bacteria in conventional and mechanicalsamplesranged from 5. 2×10^7cfu/g to 4.5×10^8cfu/g and 5. 4×10^7cfu/g to 1.35×10^8cfu/g respectively (Table 1.0). Similar study byAdegbehingbe [6] also reported a total bacterial count of(3.43×10^6 to 5.3×10^6 cfu/g)in*fufu*sampleswhich exceeded the acceptable limit of 1.0×10^5cfu/g for ready to eat food [9][10].There was no significant difference (*P=.05*) in the bacterial load of both conventional and mechanically processed food. This study indicates that the bacterial load of both conventional and mechanically processed *fufu* were above the required standards. Annan et al. (2018) also reported an unsatisfactory bacterial loadof (6.8×10^5-2.2×10^6) in conventionally processed *fufu* and a borderline bacterial loadof(3.4×10^3-4.3×10^4 cfu/g) for mechanically processed *fufu*.The processing technique, the quality of the water utilized in the production process, and the duration of exposure during sale could all be contributing factors to the discrepancy in the microbial count between these investigations. The findings of the study indicated high levels of mesophilic bacterial contamination in the water samples used by both conventional and mechanical *fufu* service providers. The detected levels of contamination (9.7×10^6cfu/ml – 1.35×10^9cfu/ml and 1.53×10^6cfu/ml – 6.3×10^8cfu/ml)for the respective conventional and mechanical processingmethodsexceed the safe and acceptable limits for mesophilic bacteria (5.0×102cfu/ml) in portable water .This confirms that the quality of water used during the preparation of *fufu* was a source of contamination. There was no statistically significant difference (*P=.05*) in the bacterial load of water used for both conventional and mechanically processed *fufu*, although the bacterial load in the water used by the conventional method was higher than that of the mechanical process (Table 1.0).

Table 1.0Total aerobic count of *fufu* and water samples from conventional and mechanical processingmethods

Vendors	*Fufu* samples(cfu/g)	Water Samples(cfu/g)

	Conventional	Mechanical	Conventional	Mechanical
1	$9.4x\ 10^{7}\pm0.8^{a}$	$1.3x\ 10^{8}\pm0.1^{d}$	$1.0x\ 10^{9}\pm0.2^{c}$	$1.5x\ 10^{6}\pm0.3^{a}$
2	$5.2x\ 10^{7}\pm0.3^{a}$	$1.1x\ 10^{8}\pm0.3^{c}$	$9.4x\ 10^{7}\pm0.5^{ab}$	$7.0x\ 10^{7}\pm0.2^{b}$
3	$8.2x\ 10^{7}\pm0.1^{a}$	$7.8x\ 10^{7}\pm0.2^{b}$	$1.3x\ 10^{8}\pm0.1^{b}$	$6.3x\ 10^{8}\pm0.5^{c}$
4	$4.5x\ 10^{8}\pm0.3^{c}$	$6.3x\ 10^{7}\pm0.1^{a}$	$4.3x\ 10^{7}\pm0.2^{a}$	$9.3x\ 10^{7}\pm0.1^{b}$
5	$3.7x\ 10^{8}\pm0.4^{b}$	$5.4x\ 10^{7}\pm0.3^{a}$	$5.2x\ 10^{7}\pm0.4^{a}$	$1.1x\ 10^{7}\pm0.2^{a}$

Data expressed as mean ±SD.
Means bearing different superscripts in the same column are significantly different (*P=.05*).

Total Coliform Counts

The total coliform counts of the *fufu* samples ranged from 6.1×10^{6} cfu/g to 3.7×10^{7}cfu/g for conventionally produced *fufu* and from 6.5×106 cfu/g to 5.6×10^{7}cfu/gfor mechanically produced *fufu*(Table 2). The total coliform counts of the fufu samples werehigher than the required amount of $1.\ 0\times10^{2}$cfu/g [9].There was a statistically significant difference between total coliform counts of the *fufu* samples produced conventionally and those produced mechanically(*P=.05*).This results was similar to work done by Annan et al.,[2], who reported the total coliform count of $1.\ 4\times10^{3}$- 3.1×10^{4} cfu/gand 9.7×10^{1}-4.1×10^{2} cfu/g for conventionally and mechanically produced *fufu* respectivelyalso above the acceptable limit. Akoma [11] also reported the presence of *Klebsiella* sp. and *Pseudomonas* sp. in *fufu* samples. The presence of coliforms in food is used as a hygiene indicator; thustheir presence is an indication of poor hygiene practices during food preparation[12].This indicates that all the *fufu* samples collected during the study are hazardous and unsanitary for human consumption. The detected coliform contamination levels were 5.3×10^{6}cfu/ml – 1.28×10^{8}cfu/ml for the water samplesfrom conventional *fufu* and 6.9×10^{6}cfu/ml – 3.5×10^{7}cfu/ml for the water used for mechanical *fufu* production. Although the coliform load in the water samples used for the fufu samples prepared by the mechanical methods were lower than that of the conventional, there were no statistically significant difference between them (*P=.05*).

Table 2. Total Coliform counts of *fufu* and water samples from conventional and mechanical processingmethods

Vendors	***Fufu* samples (cfu/g)**		**Water Samples(cfu/g)**	
	Conventional	Mechanical	Conventional	Mechanical
1	$9.5x\ 10^{5}\pm0.1^{a}$	$3.5x\ 10^{7}\pm0.6^{a}$	$5.3x\ 10^{6}\pm0.3^{a}$	$6.9x\ 10^{6}\pm0.5^{a}$
2	$6.3x\ 10^{6}\pm0.4^{a}$	$5.6x\ 10^{7}\pm0.2^{b}$	$1.2x\ 10^{8}\pm0.2^{b}$	$3.6x\ 10^{7}\pm0.3^{d}$
3	$3.7x\ 10^{7}\pm0.5^{b}$	$4.7x\ 10^{7}\pm0.4^{c}$	$4.5x\ 10^{7}\pm0.5^{c}$	$1.0x\ 10^{7}\pm0.2^{bc}$
4	$8.4x\ 10^{6}\pm0.2^{a}$	$6.5x\ 10^{7}\pm0.3^{d}$	$3.9x\ 10^{7}\pm0.3^{c}$	$1.1x\ 10^{7}\pm0.1^{c}$
5	$6.0x\ 10^{6}\pm0.4^{a}$	$6.6x\ 10^{6}\pm0.1^{e}$	$1.2x\ 10^{7}\pm0.4^{d}$	$8.7x\ 10^{6}\pm0.3^{ab}$

Data expressed as mean ±SD.
Means bearing different superscripts in the same column are significantly different (*P=.05*).

Faecal *Coliform*(*Escherichia coli*)Contamination of *Fufu*

The faecal coliform (*Escherichia coli*) in the conventionally pounded *fufu*(4.5×10^{4} to 5.8×10^{6}cfu/g) were higher than that of the mechanically milled *fufu*($9.\ 4\times10^{4}$ to 4.7×10^{6}cfu/g).There was no statistically significant difference between the faecal coliform of

both conventioanlly processed *fufu* and mecchanically processed ones(*P=.05*).The findings of this study was similar to thework done by Annan et al., [2], who also reported similar trends.The detected levels of faecal coliforms in the *fufu* samples from both conventional and mechanical processed *fufu* were significantly high and exceeded the safe and acceptable limit of 20 - <100cfu/g [10]. The findings of the study also indicated the presence of some faecal coliforms in the water used by both methods (conventional; 4.1×10^5cfu/g – 8.4×10^6cfu/g and mechanical 6.3×10^4cfu/g and 7.5×10^6cfu/g). However, some *fufu* milling machine operators recorded nofaecal coliform contamination in both *fufu* and water samples which is indicative of relatively better hygienic conditions and relatively good water quality at those facilities(Table 3).

Table 3: Feacal Coliforms(*Escherichia coli)*countsin *fufu* and water samplesfrom conventional and mechanical processing methods

Vendors	*Fufu* samples (cfu/g)		Water Samples (cfu/g)	
	Conventional	Mechanical	Conventional	Mechanical
1	$4.4x\ 10^5\pm0.5^a$	0.0	$4.4x\ 10^5\pm0.6^a$	0.0
2	$5.8x\ 10^6\ \pm0.3^b$	0.0	$8.4x\ 10^6\pm0.5^b$	$6.8x\ 10^6\pm0.4^b$
3	$5.3x\ 10^6\pm0.2^b$	$9.4x\ 10^4\pm0.2^a$	0.0	0.0
4	$4.5x\ 10^4\pm0.6^a$	$4.7x\ 10^6\pm0.4^b$	0.0	$7.6x\ 10^6\pm0.2^c$
5	$2.0x\ 10^5\pm0.5^a$	$4.4x\ 10^6\pm0.5^b$	$4.1x\ 10^5\pm0.4^a$	$6.3x\ 10^4\pm0.3^a$

Data expressed as mean ±SD.
Means bearing different superscripts in the same column are significantly different (*P=.05*)

Contamination of *Fufu* and Water by *Salmonella typhi*

The results of the *Salmonella* test in this study showed that there were no Salmonella bacteria found in any of the *fufu* and water samples. This research shows that the machines, tools, and water used in making *fufu*are free from*Salmonella* bacteria; hence *fufu* consumers in the metropolis may not face treat of salmonellosis and typhoid from it consumption.

Contamination levels of *Staphylococcus aureus*in*fufu* and water

Conventionally processed *fufu* recorded *Staphylococcusaureus* count ranging from $5.7\ x\ 10^4$ -$5.5\ x\ 10^5$ cfu/g and mechanically processed *fufu* recorded *Staphylococcus aureus*count from $9.5x\ 10^3$ - $5.9x10^5$ cfu/g. There was no statistically significant difference in the *Staphylococcus aureus*count betweenthe conventionally and mechanically processed *fufu*(*P=.05*).This differsfromthe study carried out by Annan et al., [2] who describe the *S. aures* count of traditionally processed *fufu* ($1.5x10^2$-$2.0\ x10^3$cfu/g) and mechanically processed ones (0-$8.9\ x10^2$cfu/g) as borderline (20-$\leq10^4$) .Another study by Akoma [11]and Ewanfo [13]reported the presence of *Staphylococcus aureus* in *fufu* samples in Lokoja and Benin market respectively.Food poisoning can occur when a food handler contaminates food, or from surfaces and equipment that the food comes into contact with. *Staphylococcus aureus* is a common bacteria that can be found on the skin, hair, noses, and throats of people and animals [2].Furthermore, the storage conditions of the fufu samples favors the rapid growth of the *Staphylococcus aureus*to levels ($>10^5$ cfu/g) which initiates toxin production [14]. *Staphylococcus aureus* in both conventional and mechanical *fufu* samples are classified as unsatisfactory ($>10^4$cfu/g), however, *fufu* that was mechanically processed had a higher microbiological load than *fufu* that was made using theconventional method.The amount of *Staphylococcus aureus* in water used for conventional and mechanical processing ranged from 3.8×10^3cfu/g to 3.2×10^5cfu/g and 3. 4×10^3cfu/g to 6.4×10^4cfu/g respectively. *Staphylococcus*

aureus in the water used for conventional processed *fufu* are classified as unsatisfactory ($>10^4$cfu/g) whereas the *Staphylococcus aureus*count in mechanically processed *fufu* are classified as borderline (20-$\leq 10^4$ cfu/g)[10]. There was no statistically significant difference in the *Staphylococcus aureus* count of water used for conventionally and mechanically processed *fufu*.(*P=.05*).

Table 4. Levels of *Staphylococcus aureusfufu* and water samples

Vendors	*Fufu* samples (cfu/g)		Water Samples (cfu/g)	
	Conventional	Mechanical	Conventional	Mechanical
1	$3.2x\ 10^5 \pm 0.4^{ab}$	$1.1x\ 10^5 \pm 0.1^{c}$	$9.3x\ 10^4 \pm 0.2^{b}$	$3.5x\ 10^4 \pm 0.5^{ab}$
2	$5.7x\ 10^4 \pm 0.2^{a}$	$5.9x\ 10^5 \pm 0.3^{d}$	$3.2x\ 10^4 \pm 0.5^{a}$	$6.9x\ 10^4 \pm 0.2^{b}$
3	$1.1x\ 10^5 \pm 0.3^{a}$	$1.2x\ 10^5 \pm 0.2^{c}$	$3.2x\ 10^5 \pm 0.1^{c}$	$3.4x\ 10^3 \pm 0.3^{a}$
4	$5.5x\ 10^6 \pm 0.2^{c}$	$7.0x\ 10^4 \pm 0.1^{b}$	$3.3x\ 10^4 \pm 0.2^{a}$	$4.1x\ 10^5 \pm 0.1^{c}$
5	$4.6x\ 10^5 \pm 0.3^{b}$	$9.5x\ 10^3 \pm 0.3^{a}$	$3.8x\ 10^3 \pm 0.4^{a}$	$6.3x\ 10^4 \pm 0.2^{b}$

Data expressed as mean ±SD.
Means bearing different superscripts in the same column are significantly different (*P=.05*)

Contamination levels of Yeast and Moulds in *fufu* and water

The recorded yeast counts exceeded the acceptable limitof 1.0×10^3cfu/g in both *fufu* (Conventional:8.1×10^5cfu/g-8.3×10^6cfu/g, mechanical 1.3×10^3cfu/g-5.8×10^6cfu/g) and water samples (Conventional: 8.5×10^6cfu/g-4.6×10^7cfu/g, mechanical 9.4×10^2cfu/g-6.5×10^6cfu/g) obtained from the vendors using both conventional pounding and mechanized milling (Table 5 and 6). There was no statistically significant difference in the yeast count of the *fufu* from the traditional and mechanized methods(*P=.05*) however, there was a statistically significant difference in the yeast count of the water from the traditional and mechanized methods(*P=.05*).The outcome of the assay indicated only 1(10%) of the *fufu* samples recorded mould colonization with mean count of 7.0×10^1cfu/g which came from a sample from the mechanized mills. There was no statistically significant difference in the mould count of the *fufu* from the traditional and machine methods, however, there was a statistically significant difference in the mould count of the water from the traditional and machine methods. The amount of moulds found in the *fufu* and water used for processing was lower than the safe limit of $1.\ 0\times10^3$cfu/g. The ability of moulds to generate spores makes them ubiquitous environmental pollutants. Their appearance in the *fufu* samples utilized in this investigation may have been due to this [6].

Table 5. Contamination levels of yeast in *fufu* and water from conventional and mechanized processing

Vendors	*Fufu* samples (cfu/g)		Water Samples (cfu/g)	
	Conventional	Mechanical	Conventional	Mechanical
1	$1.0x\ 10^6 \pm 0.5^{a}$	$5.8x\ 10^6 \pm 0.1^{d}$	$3.4x\ 10^7 \pm 0.3^{b}$	$9.4x\ 10^1 \pm 0.3^{a}$
2	$1.1x\ 10^6 \pm 0.2^{a}$	$4.5x\ 10^4 \pm 0.3^{a}$	$4.6x\ 10^7 \pm 0.5^{c}$	$3.6x\ 10^5 \pm 0.2^{b}$
3	$4.4x\ 10^6 \pm 0.1^{b}$	$1.3x\ 10^3 \pm 0.2^{a}$	$3.9x\ 10^7 \pm 0.1^{bc}$	$5.3x\ 10^5 \pm 0.5^{b}$
4	$8.3x\ 10^6 \pm 0.3^{c}$	$9.5x\ 10^5 \pm 0.1^{b}$	$1.1x\ 10^7 \pm 0.2^{a}$	$6.5x\ 10^6 \pm 0.1^{c}$
5	$8.1x\ 10^5 \pm 0.3^{a}$	$4.8x\ 10^6 \pm 0.3^{c}$	$8.4x\ 10^6 \pm 0.4^{a}$	$1.1x\ 10^6 \pm 0.3^{d}$

Data expressed as mean ±SD.
Means bearing different superscripts in the same column are significantly different (*P=.05*)

Table 6. Contamination levels of mould in *fufu* and water from conventional and mechanized processing

Vendors	*Fufu* samples (cfu/g)		Water Samples (cfu/g)	
	Conventional	Mechanical	Conventional	Mechanical
1	0.0	0.0	0.0	$9.3x\ 10^{1}\pm0.3^{a}$
2	0.0	0.0	$2.8x\ 10^{1}\pm0.3^{c}$	$3.1x\ 10^{1}\pm0.2^{b}$
3	0.0	0.0	0.0	$3.5x\ 10^{2}\pm0.5^{c}$
4	0.0	0.0	$2.3x\ 10^{1}\pm0.2^{a}$	$4.7x\ 10^{2}\pm0.1^{d}$
5	0.0	$7.0x\ 10^{1}\pm0.2$	$6.0x\ 10^{1}\pm0.3^{b}$	$1.5x\ 10^{2}\pm0.3^{e}$

Data expressed as mean ±SD.
Means bearing different superscripts in the same column are significantly different (*P=.05*)

Conclusion

The research has demonstrated that *fufu* prepared by both conventional and mechanized process within Kumasi metropolis contain high microbial load which exceeds safe and acceptable standards and are unsafe and unwholesome for human consumption. *Escherichia coli*, and *Staphylococcus aureus* were detected in the *fufu* samples.It is worthy to note that most vendors serve *fufu* with hot soup which has the potential oflowering the microbial load of the fufu, however if the soup used is cold, it can contribute to microbial load which will be detrimental to the health of consumers. The water used in the preparation of the *fufu* also failed the microbial tests and were classified as unsafe with total aerobic and coliform counts exceeding the acceptable limits. The water samples were also contaminated with *Escherichia coli* and *Staphylococcus aureus* with counts exceeding the safe and acceptable limit. Although both the conventional and mechanized processing had high microbial load, the microbial count in the mechanized processing were lower than the conventional processing method.As a result, when processing *fufu*, appropriate hygienic conditions and good preparation practices are required.

Conflicts of Interest

The authors declare there are no conflicts of interest.

Authors' contributions

'Francis Xavier Tebri' designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. 'Doreen Dedo Adi[1]'supervision, drafting of manuscript, editing, analysis. 'Yaa Mawufemor Akubia , and Abena Sekyere' edited the work. All authors read and approved the final manuscript.

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