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Evaluation of the Microorganism Present in Garri Sold within Local Market and Garri Producers in Ozoro Community

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

This study evaluated the microorganisms (bacteria and fungi) present in garri sold within local market and garri producers at Ozoro Community. This was achieved through microbial analysis of samples to determine microbial count and strains available. Contamination of sample was observed for garri sold at different locations with the bacteria count seen to be higher at the local market 1 ($42x10^6$cfu/10g) and garri producer ($41x10^6$cfu/10g) than at local market 2 ($38x10^6$cfu/10g) which shows that market activity may not be the contamination source. Bacteria isolates indicated the presence of pathogen associated with disease and food poisoning syndrome such as *E. coli, Staphylococcus, Bacilli* and *Pseudomonas. Staphylococcus* and *Escherichia coli* were observed to be the dominant strains of microbes in garri sold across all assessed location which poses health

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risk due to their pathogenicity. Fungi were detected in garri samples assessed from all three locations with an increasing trend from producer to sales point which is an indication that garri contamination with fungi must have occurred from post production activities in the market and sales point. The fungi isolates obtained from the samples are*Asperillus flavus Penicilum spp.and Candida albican. Asperillus flavus andPenicilum spp.* were observed to be the dominant strains of microbes in garri sold across all assessed location. The samples of garri sold around Ozoro are unsafe since they did not meet with the recommended limit by Food and Agriculture Organization (FAO) of $1x10^4$cfu/g for bacteria count and $1x10^4$cfu/g for fungi count and *E.coli* count of 0. Hygienic practices should be strictly adhered to prevent contamination of garri produced.

Keywords: Evaluation; garri; market; microorganism; ozoro; producers.

1. INTRODUCTION

Nigeria continues to rely heavily on cassava as a food crop because of its many applications. With edible tuberous roots and leaves that are consumed in some regions of the world, cassava is primarily farmed in tropical countries [1]. Cassava can be eaten in a variety of ways, including as boiled, roasted, or fried, and is typically treated before consumption to preserve and detoxify it. In Nigeria, cassava is widely recognized as the primary food product of national importance, utilized in the preparation of garri, lafun, starch, and fufu. Cassava tubers contain high amount of carbohydrate which makes it a high demand food especially for the lower income earners based on its cheap cost of acquisition [2].

Most cassava is processed into many products. According to Olopade *et al*. [3] Garri makes up 70% of Nigeria's total cassava production and is the most widely consumed product among cassava varieties. The way each processing and handling step is handled determines the final product's quality. Both in homes and factories, the process of turning cassava into garri typically takes three to five days, with an average moisture content of between 8 and 14% [1]. In 2008, Huch *et al.,* [4]. observed that the variety of microbial populations that come in contact with a garri depends on the nutritional status, pH, water content as well as the nature of the organism. These microorganisms can cause deterioration in its quality and spoilage as well as other serious food borne illnesses [5].

The high consumption rate of garri makes its freedom for biological contaminants a necessity. According to Okolie *et al.,* [6] garri should be safe and suitable for human consumption, and free from abnormal flavors, odors, and living insects. From the view point of food safety, garri itself may not constitute health hazard, since it has been estimated that various operation involved during processing it usually results its total cyanide concentration. However, hygiene practices have raised new concerns due to the health impact of microbial agents [1].

The processing of cassava into garri usually takes three to five days both at household and factory levels and its average moisture content is about 8-14 percent. Whichever method is adopted, the most important thing is to maintain stringent hygienic measures especially between the period of finishing and consumption of food [7]. Since it has been calculated that different processing operations often resulted in a reduction in its overall cyanide level, garri itself may not constitute a health threat [8]. However, the methods used to turn cassava into garri and then sell it are primarily manual and done by women and children, exposing the product to a variety of anthropogenic contaminating factors. Research conducted in 2015 by Adejumo *et al*. found that garri that had been processed and sold in a marketplace was contaminated with germs, with a total heterotropic bacterial count of 7.75 x 103cfu/g.

Although garri should be safe for ingestion by humans, it is frequently discovered to contain biological agents such *Aspergillus, Penicillium, Fusarium, Rhizopus, Cladosporium*, and *Mucor* spp. Dust produced by breezes, storms, passing cars, and any other type of air movement that brings solid particles and heavy metals into garri is one of the main sources of contamination of the grain. As a result, the product puts people at serious risk [8].

Between the last step of manufacture and the time, unsanitary handling and procedures may potentially be the source of these contaminations. Research has shown that this kind of contamination can happen in marketplaces where a large number of people shop, which could have detrimental effects on public health due to the increased risk of

contamination by organisms of epidemiological significance [1].

As highlighted by Orji *et al.,* [9] these microorganisms can cause deterioration in food quality and spoilage, serious food borne illnesses and may pose a threat to public health. Very little attention has been given to the level of risk associated with this product especially within student environment and local market area.

This study is important as it will help to evaluate the state of produced garri in relations to microbial count. It will also help to indicate the risk associated with consumption of garri within the local market. In addition, it will help to indicate the associated sources and the likely contaminating source of garri and the overall efficiency of the production process.

2. MATERIALS AND METHODS

This study was achieved through the adoption of different scientific staged processes as showed by Orji et al., [9]

2.1 Study Area

The study area lies between latitude 5°33'N and longitudes 5°47'E. Ozoro is characterized by two climatic seasons (rainy and dry season). During the rainy season (between April and October), Ozoro usually experienced high water table levels throughout the entire community, and seasonal flooding in most parts of the community. However, the soil is only subject to mild environmental pollution associated with petroleum products release due to anthropogenic activities. The area is also home to agricultural activities such as poultry farming which generates huge amount of poultry waste. The total experimental area used for this study is the local market.

2.2 Collection of Sample/Preparation

The grab method of sampling was employed in the collection of garri samples within local market in Ozoro and garri producers. These samples were stored in a sterilized sample container prior to laboratory analysis.

2.3 Methods

2.3.1 Microbial analysis

All apparatus, materials and growth medium will be sterilized using an autoclave. Ten gram (10g) of each sample of garri was homogenized in 9ml of sterile distilled water (10^{-1} dilution). Further serial dilution of sample homogenate to 10^{-5} was carried out also in sterile distilled water, transferring 1ml of initial suspension into subsequent tubes used for the serial dilution. 0.1ml aliquot of appropriate dilution (10^{-5}) was spread on plates of MacConkey agar for coliform count. All culture plates were incubated at 37°C aerobic for 24hrs.

2.3.2 Bacteria identification and gram staining

Identification of the isolates using phenotypic characteristics was based on the various test carried out using Bergey's manual of systemic.

1ml of samples was added to test tubes containing lactose broth inverted and incubated at 37°C for 24-48hours. Tubes showing gas production and/or colour change of dye were reported as presumptive coliform test positive. The number of positive tubes in each set was combined and arranged orderly. These positive tubes were streaked out on duplicate plates of Eosin methylene blue (EMB) agar for confirmatory test. The plates were incubated for 24hrs at 37°C. Colonies from confirmatory test was gram stained and inoculated into lactose broth for completed coliform test.

Smear of bacteria culture was made on clean glass slide, air dried and heated. Smear was covered with crystal violet for 30seconds, washed with distilled water and covered with iodine solution for 60seconds. Slide was again washed with 95% ethyl alcohol and distilled water. Again the smear was covered with safranin for 30secnds, washed with distilled water and blot dried. The air dried slide was then viewed under the microscope.

2.3.3 Motility test

Sterilized wire loop was used to make a drop of the test organism on a clean slide. Three drops of peptone water were then added and the slide was covered with a slip and examined microscopically under 45X objective.

2.3.4 Catalase test

Nutrient agar medium was prepared and poured into culture tubes and flasks before sterilization. The agar slants were then incubated with organisms and an inoculated slant was kept as a

control. The cultures were incubated at 35^{0}C and 3-4 drops of the hydrogen peroxide was added on the growth of each slant culture. The culture was then observed for the appearance or absence of gas bubbles. Active bubbling shows positive catalase and absence shows negative catalase.

2.3.5 Oxidase test

A piece of filter paper was divided into three equal sections and labeled with the name of organisms. a loop full of the culture was rubbed on the moisture filter paper using a sterile loop. The colour of the smear was checked exactly 15-30seconds after rubbing the cell on the reagent moistened filter paper. A deep blue color indicates positive reaction. Light violet or purple color which developed within 10 seconds was recorded.

2.3.6 Indole production test

1% tryptone broth was prepared and sterilized using autoclave at 151bs for 15 minutes. The trytone broth was inoculated with test organism and an uninoculated tube was kept as control. The tubes were inoculated at 35^{0}C for 48hours; 1ml of Kovac s reagent was added after 48hours of inoculation. The tubes were then shaken at intervals of 10 minutes and allowed to stand to permit the reagent to come to the top. The tubes were then observed for cherry rod layers with red surfaces indicating positive indole.

2.3.7 Starch hydrolysis test

Starch agar was prepared and sterilized using an autoclave at 151bs for 15minutes. The medium was poured into a petri plate and allowed to solidify and test organisms were inoculated on to the plate with a sterile loop. The plates were then incubated at 35^{O}C for 48hours, flooded with grams iodine and observed for clear zone around the test organism.

2.3.8 Hydrogen sulphide test

SIM agar was prepared and sterilized using an autoclave. The agar tubes were then labeled with the name of the organisms to be inoculated and inoculated appropriately. The tubes were then incubated at 35^{O}C to 36^{O}C for 48hours and observed for the presence of black coloration along the line of inoculation.

2.3.9 Coagulate test

A drop of distilled water was placed on two slides and colony of test organism emulsified on each slide to make a thick suspension. A loopful of plasma was then added to one of the suspension and mixed gently. This was observed for clumping of the organism within 10seconds. Clumping of the organism indicated positive reaction.

2.3.10 Citrus utilization test

Simmon's citrate agar medium was prepared and sterilized using an autoclave. 5ml of media was hence poured into the culture tube and agar slants were prepared and inoculated with test organisms. The uninoculated tubes were kept as control and all tubes incubated at 37^{O}C for 48hours. Slant cultures were hence observed for growth and coloration of the media.

2.3.11 Methyl-red

MRVP broth was prepared and sterilized using an autoclave and 5ml of broth poured into separate tubes. Tubes were then inoculated with test organisms and incubated at 25Oc for 48hours. 5 drops of methylred indicator was added to the tubes of each set and change in colour was observed for MR test.

3. RESULTS AND DISCUSSION

3.1 Results

Average results from evaluation of microorganism present in Garri sold within the local markets in Ozoro are represented below;

Sample A = Local Market 1
Sample B = Local Market 2
Sample C = Garri producer

3.2 Bacteria Content of Garri Sample

The bacteria isolates indicated the presence of *E. coli, Staphylococcus, Bacilli and Pseudomonas.*

3.3 Fungi Content of Garri Sample

The Fungi Isolates obtained from the samples are *Asperillus flavus Penicilum spp.and Candida albican.*

Table 1. Microbial count present in assessed sample of garri

Parameters	Sample A	Sample B	Sample C	FAO Limit
Bacteria Count x10^4 (cfu/10g)	42	38	41	1 x 10^4
Fungi Count x10^3 (cfu/10g)	30	33	27	1 x 10^2

Table 2. Microbial isolates present in assessed sample of garri

Parameters	Frequency	Percentage Occurrence
Staphylococcus aureus	2	17
Escherichia coli	2	17
Bacillus sp.	1	8
Pseudomonas sp.	1	8
Asperillus flavus	2	17
Penicilum spp.	2	17
Candida albican	1	8

Table 3. Characteristics of bacteria isolated from sample of garri

Species Name	Morphological Test		Biochemical Test								
	Gram Stain	Shape	Motility	Catalase	Coagulase	Oxidase	Spore	Indole Production	H_2S Production	CU	SH
Pseudomonas sp.	-	Rod	-	+	-	+	-	-	-	-	-
Staphylococcus aeurus	+	round	-	+	-	-	-	-	-	-	-
Bacillus sp.	+	Rod	+	+	-	-	+	-	-	-	+
Echerichia coli.	+	Rod	+	+	-	-	+	-	-	-	+

Table 4. Characteristics of fungi isolated from sample of garri

Organism	Sample	Shape	Surface	colour	septation	Reproduction
Aspergillus sp.	Raw	C	Powdery	Brown	Septate	Sexual

4. DISCUSSION

Samples of garri obtained from the various locations showed that garri sold across all locations were contaminated with bacteria. These contaminations can be reported to have occurred through processing environment, processing water and public display of this food item. From Table 2, the bacteria count was however higher at the local market 1 (42x10^6cfu/10g) and garri producer (41x10^6cfu/10g) than at local market 2 (38x10^6cfu/10g) which shows that market activity may not be the contamination source. Bacteria isolates indicated the presence of pathogen associated with disease and food poisoning syndrome such as E. coli, staphylococcus, bacilli and pseudomonas. It was observed that the microbial counts increased generally from processing areas (factories) to the local market 1 but not at the local market 2 which can be attributed to exposure of garri to sunlight which may have cause death to microbial cells in local market 2. Study by Adejumo *et al.,* [1] observed a similar result through microbiological quality assessment of garri in Ibadan which revealed the presence of *E. coli, Staphylococcus, Bacilli and Pseudomonas*. *Staphylococcus aureus* and *Escherichia coli* were observed to be the dominant strains of microbes in garri sold across all assessed location which poses health risk due to their pathogenicity. These samples did not meet with recommended limit by the Food and Agriculture Organization (FAO) of 1 x 10^4cfu/g and *E.coli* count of 0 which makes it unsafe for consumption.

Fungi were detected in Garri samples assessed from all three locations with an increasing trend from producer to sales point which is an indication that garri contamination with fungi must have occurred from post production

activities in the market sales point. The fungi isolates obtained from the samples *Aspergillus sp.* and *Penicillium sp.* which are common environmental contaminants usually seen in the contamination of ready-to-eat foods as also observed by Akoma *et al.,* [10]. The presence of fungi in garri sample calls for concern since environmental conditions are likely to promote their growth. The high fungi count observed can also be attributed to long storage period as the fungi count were significantly higher than the fungi count of 2.46 - 2.59cfu/g values observed by Akoma *et al.,* [10]. This result however coincides with high fungi count observed by Isichei-Ukah and Imiere [8] which was attributed to traditional methods of processing and packaging, improper holding temperature and poor personal hygiene of food handlers. *Asperillus flavus* and *Penicilum* were observed as amongst the most dominant microbes in the observed samples and may pose health implications. These samples did not meet with recommended limit by the Food and Agriculture Organization (FAO) of 1 x 10^2cfu/g for fungi count which makes it unsafe for consumption [11].

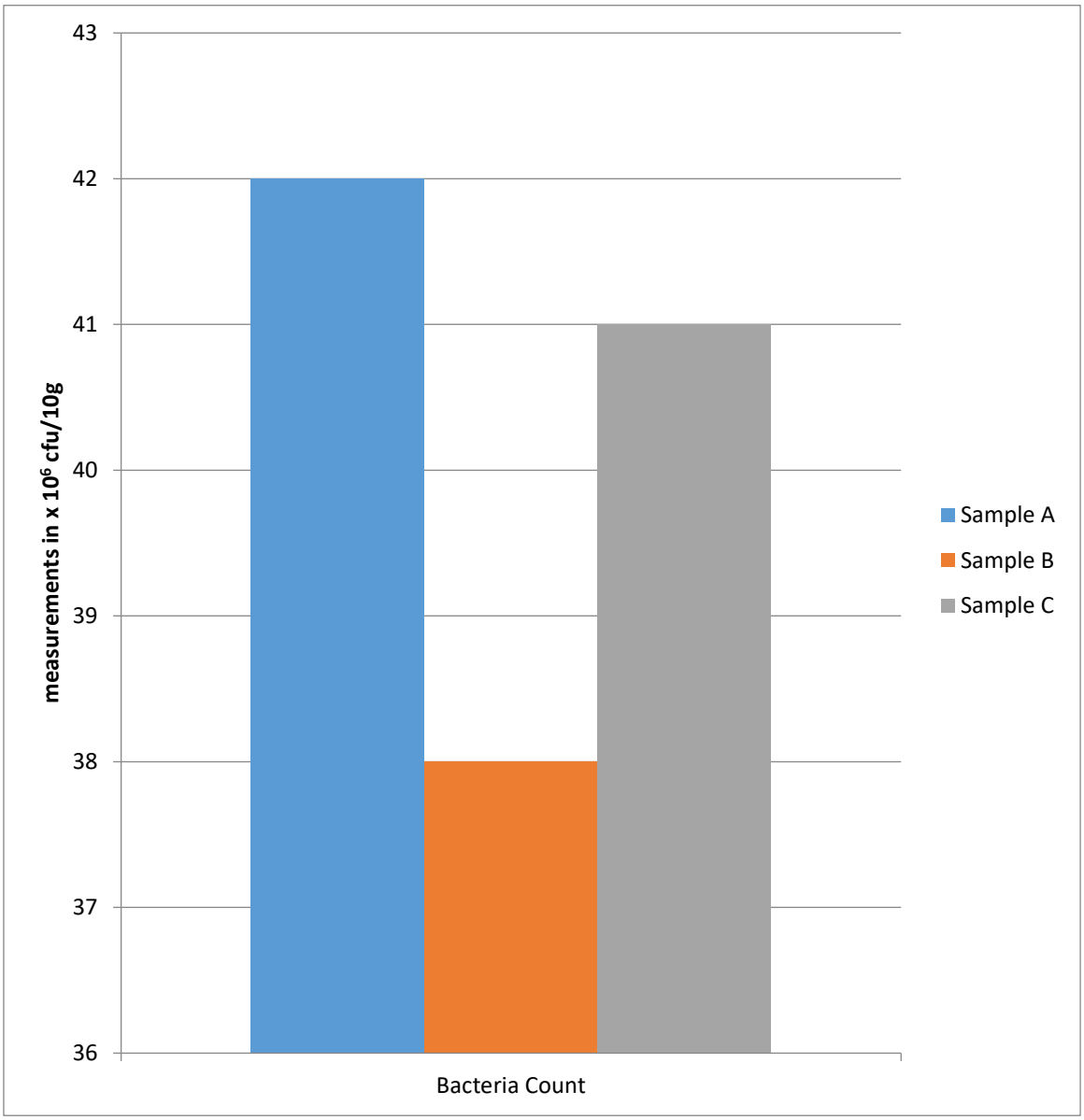

Fig. 1. Bacteria count in samples of garri assessed

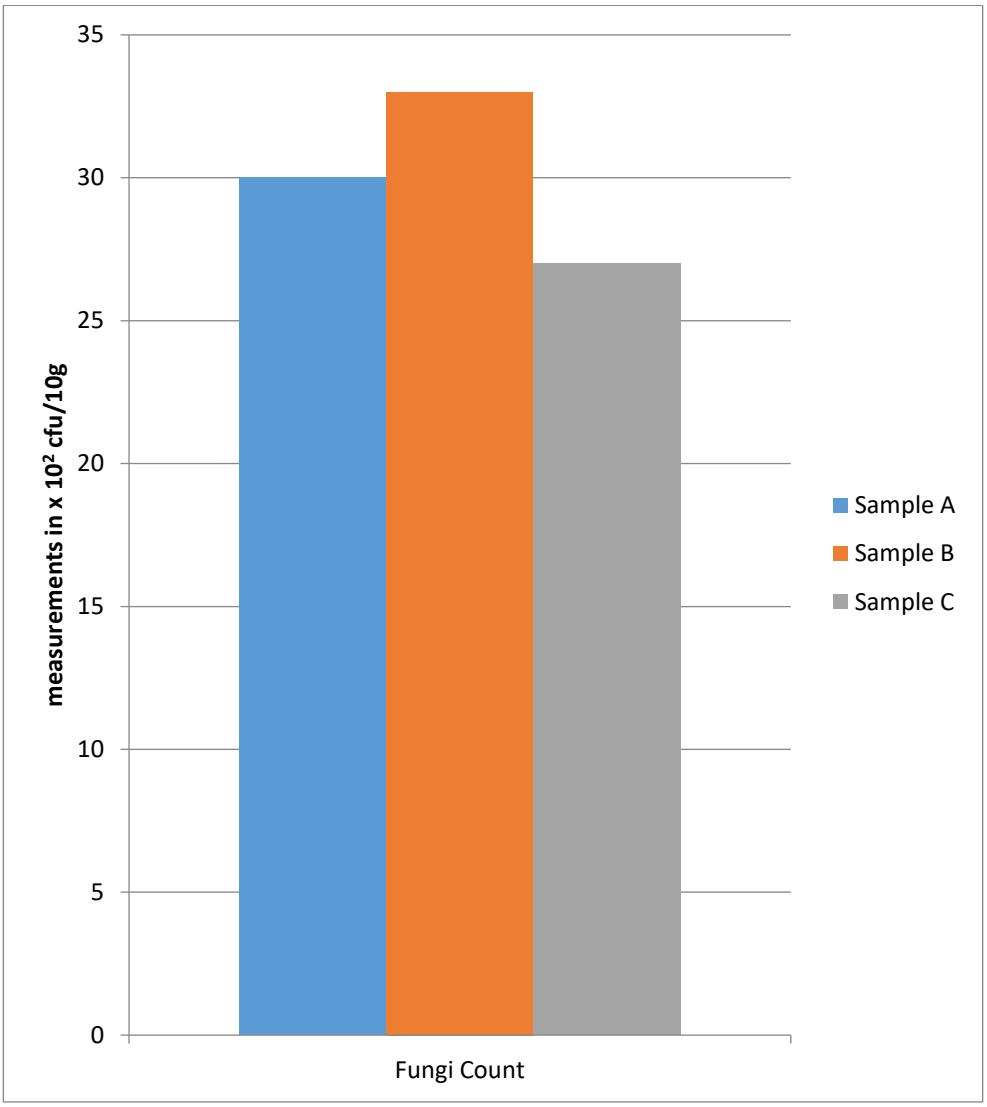

Fig. 2. Fungi count in samples of garri assessed

5. CONCLUSION

This study has shown cassava made product such as garri poses threat to human health as a result of its contamination with both bacteria and fungi species. Microbial counts were observed to be high especially for bacteria reaching up to $46x10^6$cfu/10g at vending outlets and $33x10^2$cfu/10g for fungi. Some of the isolated organisms from the samples of garri including *Staphylococcus aureus, Escherichia coli, Asperillus flavus* and *Penicillum* are well-known causes of food borne diseases and food intoxications. It can therefore be concluded that the samples of garri sold around markets in Ozoro are unsafe since they did not meet with the recommended limit by Food and Agriculture Organization (FAO) of 1 x 10^4cfu/g for bacteria count and 1 x 10^4cfu/g for fungi count and *E.coli* count of 0.

From the above conclusion, the following recommendations are made;Hygienic practices should be strictly adhered to prevent contamination of garri produced.,Exposure of garri in vending outlets should be prohibited especially in areas was there are open dumping.,Water used in garri production should be subjected to primary water treatmentOperating parameters in Garri producing outlets should be assessed to identify contaminating sources.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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