



Microbial Assessment of Selected, Locally-Fermented and Ready-to-eat Cassava Products Sold in Lokoja, Nigeria

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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 was conducted to assess the microbiological safety of locally-fermented, ready-to-eat cassava products, namely garri and 'fufu', in Lokoja. A total of sixty samples comprising; twenty white garri, twenty yellow garri and twenty fufu were subjected to microbial analysis. The samples were serially diluted to 10^{-4} and appropriate dilutions inoculated by spread plate method into Nutrient agar, MacConkey agar and Potato Dextrose agar plates which were used for total aerobic plate count (TAPC), coliform count (CC) and fungal count respectively. The TAPC for white garri ranged from 0.78 to 3.83 log cfu/g, the coliform count ranged from no growth (NG) to 3.80 log cfu/g, while the mean fungal count ranged from 1.96 to 3.39 log cfu/g. The TAPC for yellow garri ranged from 2.04 to 3.95 log cfu/g, the coliform count ranged from NG to 3.62 log cfu/g and the fungal count ranged from 2.08 to 3.44 log cfu/g. The TAPC of fufu was within the range of 1.07 to 3.70 log cfu/g, the coliform count ranged from NG to 3.48 log cfu/g and the fungal count ranged from 1.94 to 2.78 log cfu/g. The bacteria isolated include *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and *Klebsiella* spp. The fungi isolated from the study samples include *Aspergillus niger*, *Cladosporium* spp., *Fusarium*

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spp., *Rhizopus* spp., *Alternaria* spp., *Montospora* spp., and *Penicillium* spp. The pH of the samples ranged from 4.02 to 4.96 in white garri, 4.02 to 4.99 in yellow garri, and 5.02 to 6.44 in fufu. Findings showed that these widely consumed fermented (ready-to-eat) cassava products presents (may represent) a serious risk and route for transmission of food borne pathogens to consumers and generally huge economic disadvantage to food handlers. Improving manufacturing, packaging and storage practices in garri production and for public health purposes are strongly encouraged.

Keywords: Microbiological safety; cassava products; microbial assessment.

1. INTRODUCTION

Food is the basic necessity for human survival and attainment of food security is the priority of any country. However, it is important that food security should not be seen only in the perspective of availability either quantitatively or qualitatively. Therefore, microbiological safety of foods should also be given important consideration in order to protect the health of the people as the microbial-contaminated foods could serve as vehicle for the transmission of food borne diseases [1,2]. Food may be available but the source from which it is produced and / or processed may be unhygienic and even the chemicals that may be used to preserve it may cause serious health hazard [1].

Coliforms, particularly *Escherichia coli* are used as indicators of post process contamination and also the presence of *E. coli* in foods serves as an indicator of faecal contamination [3]. Coliforms are group of closely related Gram negative, non-spore forming, rod-shaped aerobes and facultative anaerobes that ferment lactose to produce acid and gas within 48 h at 35°C. They are mostly harmless and lives in soil, water and in the gut of animals with few enteric pathogens including *Salmonellae*, *Shigellae* and enteropathogenic *E. coli* [4].

Filamentous moulds and yeasts are common spoilage organisms of food products and some species of *Penicillia* and *Aspergilli* have been reported as spoilage organisms of a variety of foods on which they may produce a quite number of mycotoxins [5].

Cassava ranks fourth in the list of major crops in developing countries after rice, wheat and maize and it is used for the production of a variety of West African foods [6]. In its natural state, it is toxic to man as it may contain high levels of linamarin, a cyanogenic glucoside. Hence, processing it through fermentation enhances its detoxification, quality and safety.

Garri and fufu are the few finished products of fermented cassava in many parts of Nigeria, and

if not properly and carefully handled during processing and / or storage, it could serve as vehicle for transmission of food borne pathogens. Moreover, there could be economic losses and widespread of food borne illnesses as a result of contamination by these microorganisms. This study therefore is aimed at evaluating locally fermented ready-to-eat cassava products (garri and fufu) for any microbial contamination. Further, concerted attempt at awareness creation on food safety to handlers and consumers and relevant contributions to the body of knowledge on appropriate manufacturing, packaging and storage practices will be made.

2. MATERIALS AND METHODS

2.1 Sample Collection

A total of sixty samples of locally-fermented, ready-to-eat cassava products, comprising twenty white garri, twenty yellow garri and twenty fufu were collected aseptically from the five major markets in Lokoja Kogi state which includes; Ganaja, Adankolo, Lokongoma, Old and New International Markets. In each of the markets, four samples (i.e. from four vendors) of each of the ready-to-eat locally fermented cassava products were collected in sterile nylon bags and transported to Salem University Advanced Microbiology Laboratory for analyses.

2.2 Microbiological Analysis

Microbiological analysis was carried out according to the following conventional microbiological procedures. The analysis involved total aerobic plate count, fungal count and coliform count. This was determined by the spread plate method using standard microbiological techniques.

2.2.1 Enumeration of microbial isolates

Ten gram (10 g) of each sample were homogenized in 90 ml sterile distilled water (10^{-1} dilution). Serial dilution of sample homogenate to

10^{-4} was carried out also in sterile distilled water for colony count. Approximate 0.1 ml aliquot of appropriate dilutions were spread plated on plates of Nutrient agar and Potato Dextrose agar supplemented with 0.2 µg of chloramphenicol (all from Bio-laboratory, Hungary). All Nutrient agar and MacConkey agar plates were incubated at 37°C for 24-48 h while all potato dextrose agar plates were incubated at 25°C for 72-120 h. All plates were prepared in duplicate. Culture plates were examined, while enumeration and identification of colonies was carried out at the end of the incubation period. The total microbial population was expressed as log of colony forming unit per gram of the sample (log cfu/g).

Colonial morphology, microscopy and biochemical tests were used to identify the bacterial isolates [7] while the identification of characteristic fungal isolates were based on morphology and microscopy with reference to standard atlas and keys [8].

2.2.2 Detection of hygiene indicator organisms and specific food borne pathogens

Samples of the food product were plated on MacConkey agar, Manitol Salt agar and Salmonella-Shigella agar (Oxoid, England) after pre-enrichment in Selenite F broth and incubated at 35°C for 24-48 h, for isolation of *Escherichia coli*, *Staphylococci* and *Salmonellae* respectively.

2.2.3 Coliform test

Aliquot one gram samples were inoculated into Lactose broth in screw capped test tubes with inverted Durham tubes and incubated at 37°C for 24-48 h. Tubes showing gas production and/or color change of dye were reported as presumptive coliform test positive. These positive tubes were streaked out on duplicate plates of Eosin Methylene Blue (EMB) agar for confirmatory test and incubated at 37°C and 44°C respectively for 24 h. Growth of characteristic colonies on EMB medium represent confirmatory positive test which were Gram stained and inoculated into lactose broth for complete coliform test. Gas production and/or color change of dye plus Gram negative non-spore bearing rod represent presence of coliform [9]. At 44°C absence of growth was recorded for all the samples indicating absence of fecal coliforms [10].

2.2.4 Isolation and Identification of bacteria and fungi

Pure cultures of suspected colonies were obtained by repeated subculture on nutrient agar plates and potato dextrose agar plates for bacteria and fungi isolates respectively and stored on slants at 4°C until characterized. The isolation and identification of the bacteria were carried out using standard microbiological techniques including; Gram stain, catalase test, coagulase test, indole test, citrate test, oxidase test [11]. All fungi isolates were identified following previously described methods [12].

2.3 Determination of pH of the Locally Fermented Ready-to-eat Cassava Products

The pH which is an indicator for the multiplication of food microorganisms was determined using digital pH meter calibrated with standard buffer solutions. Ten grams of each sample were weighed and homogenized in 20ml of sterile distilled water in a beaker for 1 min. The solution decanted and the pH of the suspension measured.

2.4 Statistical Analysis

The mean and standard deviation of the total viable microbial count were subjected to statistical analysis using MS Excel and SPSS version 20. The mean microbial load log cfu⁻¹ and pH of the samples were presented in tables.

3. RESULTS AND DISCUSSION

The mean TAPC, fungal count, and coliform count results in log cfu/g and respective sample pH are shown in Table 1. The TAPC for white garri ranged from 0.78 to 3.83 log cfu/g, the coliform count ranged from no growth (NG) to 3.80 log cfu/g, while the mean fungal count ranged from 1.96 to 3.39 log cfu/g. The TAPC for yellow garri ranged from 2.04 to 3.95 log cfu/g, the coliform count ranged from NG to 3.62 log cfu/g and the fungal count ranged from 2.08 to 3.44 log cfu/g. The TAPC of fufu was within the range of 1.07 to 3.70 log cfu/g, the coliform count ranged from NG to 3.48 log cfu/g and the fungal count ranged from 1.94 to 2.78 log cfu/g. The bacteria isolated include *Bacillus* spp., *Enterobacter* spp., *Pseudomonas* spp., *Staphylococcus aureus*, *Salmonella* spp., *Escherichia coli* and *Klebsiella* spp. The fungi isolated from the study samples include

Aspergillus niger, *Cladosporium* spp., *Fusarium* spp., *Rhizopus* spp., *Alternaria* spp., *Montospora* spp., and *Penicillium* spp. The pH of the samples ranged from 4.02 to 4.96 in white garri, 4.02 to 4.99 in yellow garri, and 5.02 to 6.44 in fufu.

The result of this study was in line with the earlier reported data [10,13] however, it was slightly lower than another previously reported [14], with counts that ranged from 10^3 to 10^4 . Moreover, a study conducted in Ebonyi, Ogun and Oyo states in Nigeria reported microbial burden as high as 10^6 to 10^7 respectively [15] [16], while another related work reported a fungal count as high as 10^4 to 10^6 cfug⁻¹ [17]. The disparity in the microbial count from these studies could be as a result of the processing method, the quality of water used in the production process and the length of exposure during sale. A researcher equally observed back slopping used by some processors to reduce the length of time for fermentation to compromise the quality of the product [16], suggesting it could be the lead cause of high counts observed in their study. Meanwhile, the total aerobic plate count and fungi counts of this study samples were within the acceptable limit. Ready-to-eat foods with plate counts of $\leq 10^3$ cfu/g are within the acceptable limit while counts of 10^4 to 10^5 cfu/g are tolerable and counts that are $> 10^6$ cfu/g are totally unacceptable [18]. Probable coliform organisms were detected in most of the samples at high counts 10^2 to 10^3 cfu/g and the presence of *E. coli* calls for serious concern. It signifies poor sanitary condition and indicative of faecal contamination during the production process and / or storage of the fermented ready-to-eat food under study. It is also indicative of the potential presence of enteric pathogens and therefore makes the study samples of poor quality for human consumption.

Bacterial isolates from the study samples were *Bacillus* sp., *Enterobacter* sp., *Pseudomonas* sp., *Staphylococcus aureus*, *salmonella* sp., *Escherichia coli* and *Klebsiella* sp. (Table 2).

Most of the isolates were glucose positive, indole negative, catalase positive and a reasonable number of both Gram positives and Gram negatives (Table 3). The isolation of diverse microbial species from this ready-to-eat fermented foods did not completely agree with the earlier findings [10,13,16] as each author had dissimilarities in bacterial presence in their study samples. Most of these studies reported

the presence of *Staphylococcus aureus*, *Bacillus* sp., *Pseudomonas* sp., *E. coli* and *Klebsiella* sp. The observation of diverse bacteria isolates could be attributed to the fact that these studies was carried out at different regions and from different sample markets of which environmental conditions of the study areas could affect the distribution of organisms. Buyer's attitude towards the exposed food products in the market could also contribute to the microbial load and diversity as they touch the products with bare hands and taste it before they buy. The presence of *Salmonella* in this study calls for concern as this organism is the common cause of human food poisoning, and salmonellosis can affect all species of domestic animals and man. It is important to draw to our attention that the young, aged, stressed, debilitated and pregnant individuals are more susceptible while the immunosuppressed and those suffering from malnutrition are at risk for *salmonella* infection [19].

The presence of *Bacillus* and *Staphylococcus aureus* also calls for concern because some strains of these organisms are known to be toxigenic and often implicated in food borne intoxication [20,21]. *Bacillus* a common environmental contaminant and a spore former can withstand environmental stress and this may account for its presence in the samples. Meanwhile, *Staphylococcus aureus* is of human origin and their presence could therefore be from the food handlers, utensils and the environment. Moreover, garri and fufu is a common food widely consumed by all in Nigeria and increasing intake of it, especially dry garri as snacks or with cold water is an added practice that exposes the populace to serious health risk due to the microbial status of the product.

Fungal isolates from the samples collected were detailed in Table 4. More so, among all fungal isolates; most authors reported the presence of *Aspergillus* sp., *Fusarium* sp. and *Penicillium* sp. in their study [10,13,17], which agrees with the result of this study. Filamentous fungi are common environmental contaminants usually implicated in the contamination of ready-to-eat foods and this explains their presence in the study samples. More so, species of *Aspergillus*, *Penicillium* and *Fusarium* are known to produce mycotoxins [22,23,24] and their presence in the study samples calls for serious concern.

Table 1. Mean microbial count log cfu/g and pH of locally fermented cassava products in Lokoja

Sample Outlet		White Garri				Yellow Garri				'Fufu'			
		TAPC	CC	FC	pH	TAPC	CC	FC	pH	TAPC	CC	FC	pH
Adankolo	1	2.50±0.02	3.30±0.06	2.60±0.14	4.20	3.30±0.02	3.04±0.08	2.48±0.02	4.34	2.11±0.00	3.18±0.48	2.60±0.02	6.23
	2	0.98±0.19	3.47±0.12	2.46±0.17	4.66	2.62±0.01	2.98±0.19	2.90±0.02	4.83	2.36±0.27	2.60±0.11	2.76±0.40	6.44
	3	2.48±0.02	3.43±0.01	2.47±0.10	4.64	2.58±0.02	3.30±0.06	2.47±0.08	4.02	2.48±0.03	2.59±0.14	2.26±0.26	6.02
	4	1.25±0.69	3.48±0.01	2.59±0.17	4.91	3.30±0.03	2.59±0.12	2.60±0.02	4.33	2.48±0.02	NG	2.66±0.26	5.93
Lokongoma	1	2.25±0.03	NG	2.60±0.03	4.36	2.50±0.06	2.60±0.03	2.85±0.02	4.54	2.30±0.06	3.14±0.07	2.00±0.06	5.64
	2	2.40±0.02	3.43±0.02	2.48±0.02	4.43	2.47±0.18	NG	2.30±0.03	4.30	2.60±0.02	2.53±0.06	2.60±0.02	5.52
	3	2.98±0.11	3.30±0.03	2.48±0.02	4.22	2.67±0.01	3.15±0.02	2.70±0.01	4.49	0.89±0.83	3.30±0.03	2.59±0.13	5.75
	4	2.17±0.12	3.30±0.02	2.77±0.09	4.11	2.49±0.06	2.53±0.05	3.23±0.37	4.67	1.07±0.16	3.16±0.17	2.60±0.00	5.46
New	1	3.83±0.18	NG	3.39±0.40	4.40	2.11±0.05	3.72±0.34	2.59±0.16	4.52	2.50±0.06	3.43±0.02	2.48±0.03	5.62
	2	0.78±0.67	3.80±0.27	2.60±0.03	4.43	2.20±0.06	3.00±0.06	2.77±0.08	4.23	3.00±0.09	3.48±0.03	2.48±0.02	5.41
	3	2.58±0.03	3.30±0.08	2.47±0.15	4.59	3.95±0.07	2.90±0.12	2.95±0.03	4.12	2.64±0.06	NG	2.78±0.05	5.03
	4	2.00±0.06	3.45±0.21	2.60±0.01	4.17	2.08±0.05	2.30±0.06	2.60±0.02	4.04	3.48±0.02	3.43±0.01	2.48±0.01	5.14
Old	1	3.00±0.06	2.89±0.04	2.60±0.02	4.08	2.04±0.06	2.30±0.02	2.70±0.06	4.50	3.00±0.03	3.30±0.09	2.60±0.03	6.35
	2	2.08±0.05	3.85±0.00	2.47±0.10	4.87	2.17±0.08	2.46±0.19	2.95±0.01	4.12	2.08±0.05	3.30±0.05	2.47±0.10	6.23
	3	2.11±0.01	2.48±0.03	2.59±0.17	4.96	2.17±0.24	NG	2.70±0.01	4.73	1.90±0.43	2.47±0.10	1.94±0.34	6.10
	4	3.76±0.16	NG	2.60±0.03	4.15	3.69±0.12	2.00±0.06	2.00±0.12	4.94	3.24±0.34	NG	2.75±0.21	6.06
Ganaja	1	2.07±0.10	2.30±0.06	2.48±0.02	4.96	2.22±0.25	3.62±0.04	2.60±0.05	4.56	2.02±0.17	2.29±0.16	1.94±0.34	5.64
	2	2.28±0.07	2.24±0.34	1.96±0.26	4.02	2.10±0.14	3.49±0.06	3.44±0.27	4.15	2.15±0.21	3.22±0.38	2.45±0.21	5.43
	3	2.17±0.12	NG	2.75±0.21	4.14	2.05±0.21	1.95±0.49	2.66±0.26	4.77	2.20±0.12	2.90±0.02	1.98±0.19	5.02
	4	2.30±0.03	NG	2.00±0.12	4.25	3.68±0.19	NG	2.08±0.05	4.99	3.70±0.02	2.24±0.34	2.75±0.21	5.13

Key: TAPC = Total Aerobic Plate Count, CC = Coliform Count, FC = Fungal Count, NG = No Growth

Table 2. Distribution of bacteria in the food samples across the sampled markets

Market	Sample	Salm	Kleb	Staph	Bacillus	E. coli	Strept	Pseudo	Entero
Adankolo	White garri	+	+	+	-	-	-	-	-
	Yellow garri	-	-	+	+	-	-	-	-
	Fufu	-	+	+	-	-	-	+	-
Lokongoma	White garri	+	-	-	-	+	-	-	-
	Yellow garri	+	-	+	+	-	-	-	-
	Fufu	-	-	+	+	-	-	-	-
New	White garri	-	-	+	-	+	+	-	-
	Yellow garri	+	-	+	-	+	-	-	-
	Fufu	-	+	+	-	-	+	-	-
Old	White garri	+	-	+	+	+	-	-	-
	Yellow garri	-	-	+	-	+	-	-	+
	Fufu	+	-	+	-	-	+	-	-
Ganaja	White garri	-	-	+	-	+	-	-	-
	Yellow garri	-	-	+	-	+	-	-	-
	Fufu	-	-	+	-	-	-	+	-

Key: Salm = *Salmonella sp.*, Kleb = *Klebsiella sp.*, Staph = *Staphylococcus aureus*, Pseudo = *Pseudomonas sp.*, Entero = *Enterococcus sp.*, + = present, - = absent

Table 3. Biochemical characteristics of bacterial isolates

Bacteria found	Gram stain	H ₂ O ₂	Glucose	Citrate	Indole	Endospore	Oxidase	Coagulase	Catalase
<i>Salmonella sp.</i>	-	ND	+	-	-	-	-	-	+
<i>Klebsiella sp.</i>	-	ND	+	+	-	-	-	-	+
<i>S. aureus</i>	+	ND	+	+	-	-	-	+	+
<i>Bacillus sp.</i>	+	ND	+	+	-	+	+	ND	+
<i>E. coli</i>	-	ND	+	-	+	-	-	-	+
<i>Streptococcus sp.</i>	+	ND	+	ND	ND	-	ND	-	-
<i>Pseudomonas sp.</i>	-	-	-	+	-	-	+	-	+
<i>Enterococcus sp.</i>	+	-	+	-	-	-	-	-	-

Key: ND= Not determined

Table 4. Distribution of fungi in the food samples across the sampled markets

Market	Sample	Asper	Peni	Mold	Mucor	Rhi	Fus	Clado	Alter	Monto
Adankolo	White garri	+	-	-	-	-	-	-	-	-
	Yellow garri	+	+	+	-	-	-	-	-	-
	Fufu	-	+	-	-	-	-	-	-	-
Lokongoma	White garri	-	+	-	+	-	-	-	-	-
	Yellow garri	-	-	-	-	+	-	-	-	-
	Fufu	-	-	-	-	-	+	-	-	-
New	White garri	-	+	+	+	-	-	-	-	-
	Yellow garri	-	-	+	+	-	+	-	-	-
	Fufu	-	+	-	-	+	-	-	-	-
Old	White garri	-	-	-	+	+	-	-	-	-
	Yellow garri	-	-	-	-	-	-	+	+	-
	Fufu	-	-	-	-	-	+	-	-	-
Ganaja	White garri	+	-	-	-	-	+	-	-	-
	Yellow garri	-	-	-	+	-	-	-	-	+
	Fufu	-	-	-	+	-	-	-	-	-

Key: Asper = *Aspergillus sp.*, Peni = *Penicillium sp.*, Rhi = *Rhizopus sp.*, Fus = *Fusarium sp.*, Clado = *Cladosporium sp.*, Alter = *Alternaria sp.*, Monto = *Montospora sp.*,
+ = present, - = absent

The pH value of white garri and yellow garri ranged from 4.02 to 4.96 and 4.02 to 4.99 respectively while that of fufu ranged from 5.02 to 6.44 (Table 1). It was observed from the study that the pH values of fufu were higher than that of the garri samples. The pH values recorded in this study was within the range of those reported in related studies [10,13,16] for the garri samples. Moreover, a study conducted in Ebonyi state, Nigeria reported higher pH values of 5.47 to 6.61 [15] which is in disagreement with this study. This disparity observed could be attributed to the length of fermentation and storage time. A study reported a reduction in pH of cassava mash from 6.2 to 3.2 over a period of 10 fermentation days under ambient temperature of 28 – 32°C [25]. [17] It was also revealed from a study that the longer the storage time, the higher the pH. Another study attributed the increase in pH to be as a result of production of acidic metabolites by microorganisms during their growth and proliferation [26].

4. CONCLUSION

This study has shown that the fermented staple food under study were contaminated with both bacterial and fungal species, with presence of coliforms. Some of the isolated organisms are well-known causes of food borne diseases and food intoxications. With these findings, the important of hygiene during processing and/or storage of these food products cannot be overemphasized. It is also important that the food handlers properly covers the food product during storage and also reduce the length of its exposure in the market place.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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