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Original Article

Microbiological quality of fermented *Cassava Flakes* (Gari) sold in Yenagoa, Metropolis, Nigeria

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Abstract

The study investigated the microbial quality of *gari* (viz yellow and white) sold in five markets of Yenagoa metropolis, Nigeria. Fifteen samples of each type of *gari* were obtained from each market. Microbiological examination of the samples was carried using standard microbiological procedure. Results showed that total heterotrophic bacteria, total coliform and total fungi ranged from 3.848 to 4.973 Log cfu/g, 2.659 to 3.793 Log cfu/g and 3.371 to 3.832 Log cfu/g respectively (yellow *gari*); 4.206 to 5.206 Log cfu/g, 3.242 to 3.803 Log cfu/g, and 3.887 to 4.145 Log cfu/g respectively (white *gari*). Analysis of variance of Log coliform forming counts showed that there was no significance difference (P>0.05) among the various markets. The microbial density is within acceptable and tolerable level for ready to eat food. The bacterial species tentatively identified in both *gari* types include *Micrococcus, Klebsiella, Proteus, Bacillus* and *Corynebacterium* species, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Enterobacter aerogenes* and *E. coli*. The fungal species are *Aspergillus niger, Fusarium, Rhizopus, Mucor* and *Penicillium* species. Some of these microbes found in *gari* samples are suspected potential pathogens to human health.

1. Introduction

Cassava is one of the most fundamental root crops that are processed into variety of food products. Cassava is found in the tropical nations of the world. Presently, Nigeria is the largest producer of cassava in the world [1-4]. Cassava is a major source of carbohydrate which provides energy for more than 2 billion people in the tropics [1]. The cultivation, processing, marketing of cassava and its products is source of livelihood to several families in Nigeria especially in the rural area; hence it is a source of employment.

According Wenham [5] cited in Adebayo-Oyetoro *et al*. [6], cassava roots is perishable and several postharvest losses occur during storage probably due to high physiological and microbial activities that invade the commodity during processing as a result of bruises as well as high moisture content of the fresh cassava tubers, which facilitates microbial deterioration and unfavourable biochemical changes. Also, Kemdirim *et al*. [7] cited in Olopade *et al*. [8] stated that during the processing of cassava tuber into products like *gari* and flour, the biochemical compositions are altered.

Cassava has found application in the production of several products including cassava-bread production [9-11], flour, *gari, fufu,* livestock feeds, confectionaries, monosodium glutamate processing, sweeteners, glues, textiles, and pharmaceuticals [6]. Cassava also has huge potential for biofuel production such as bioethanol [1, 2, 12-14]. Of these, the main cassava food products of considerable domestic significance in Nigeria include *gari, lafun* and *fufu*[8].

Basically, gari is processed from freshly uprooted cassava tuber (which is creamy white in colour after peeling), then followed by peeling, washing, grating, dewatering/fermentation, seiving, frying and packaging. Slight variation exists among the garri processed in Nigeria depending on the region. One of the notable differences include fermentation period. Olopade *et al*. [8] described *gari* as a granulated and dehydrated cassava product, which are grouped according to their texture, length of fermentation, region or place where it is produced and colour impartation by the addition/non-addition of palm oil.

Gari can be consumed in several ways. This include eaten as snacks with coconut or groundnut and sugar [15], meat, roasted groundnuts, smoked fish, boiled beans, coconut, palm kernel, groundnut cake "*kwulikwuli*", and fermented maize snacks *Kokoro*, beverages and milk can be added together with cold water [8]. *Gari* can also be processed into semi-solid food with hot water called Eba and eaten with a variety of soups[15].

Cassava processing into *Gari* especially by small holders in Nigeria lack quality control. However, during frying in an open pan, heat is applied which aid in the reduction of microbial load and isolates. But during sales in markets, *gari* is displayed in basin without any formal covering as a result they are exposed to contamination including dust and microorganisms. Microbes have been severally described to be ubiquitous and tend to thrive well in unhygienic conditions. Since, *gari* is also consumed without application of heat. The microbes that may have invaded them in the busy markets call for concern especially when they are consumed without the application of heat. Studies have been conducted with regard to the microbial quality of some fermented foods such as *gari* and or lafun in some regions of Nigeria including Ogun and Oyo States[6], Ojo Oba Market, Akure, Ondo state [16], Ijebu-Igbo Central Market, Ogun State; Bodija Market, Ibadan, Oyo State and Garki Central Market, Abuja-Federal capita as territory [17] and Ota Ogun State [8]. There is little or no available literature on the microbiological quality of *gari* sold in Yenagoa metropolis which is a very humid environment in the Niger Delta region; hence the need for this study.

2 Materials and Methods

2.1 Field Sampling

White and yellow fermented *gari* was obtained from five major markets including Igbogene, Akenfa, Agudama-Epie, Tombia, and Swali in Yenagoa metropolis, Bayelsa state, Nigeria. Fifteen samples were purchased for each type of *gari* hence a total of thirty sample were purchased, three being for each type of *Gari* in a specific market. The *gari* samples were packaged in transparent polythene bag were placed in sterile Ziploc bag and transported to the laboratory for analysis within 3 hours of collection.

2.2 Sample preparation and enumeration of microbial counts

Twenty gram (20.0g) samples of *gari* were homogenized in 180.0 ml distilled and de-ionized water. Prepared Nutrient Agar, Sabauroud Dextrose Agar, MacConkey agar and *Samonella-Shigella* cooled in water bath at 45ºC was poured into the labeled sterile petri dishes. About 1.0 ml of the samples was serially diluted up to 10^{-6} with sterile water. Then, 0.1 ml was aseptically transferred into the petri dish. Thereafter, the plates were incubated inverted at 37°C for 24-48 hours for all bacteria associated growths (total heterotrophic bacteria, total coliform, total fungi and Samonella-Shigella counts) at 30°C for 3-5 days for fungi. The resultant colonies that grew on the various media were counted and expressed as colony forming units (cfu)/g of the *gari* samples and the colonies were isolated and preserved in slants for identification.

2.3 Microbial identification

The different colonies that grew on the MacConkey agar was streaked in Levine's eosin Methylene Blue (EMB) Agar and incubated at 37º C for 24 hours. Based on gram reaction of the isolates that grew on nutrient agar, positive cocci were streaked in Mannitol Salt Agar and incubated inverted at 37°C for 24 hours. The presence of yellow pigment suggests the presence of *Staphylococcus aureus,* which was confirmed by coagulase test. Similarly, the bacterial pure culture was streaked in Blood Agar the presence of swarming characteristics suggest *Proteus* species which was then subjected to biochemical test. All the various growth observed in the various medium were streaked in Nutrient Agar from where the biochemical test (gram reaction, citrate, catalase, oxidase, Indole, coagulase, motility, methyl red) were carried out using the scheme of Cheesbrough[18] and Benson [19].The scheme of Cheesbrough (2006) and Bergey's Manual of Determinative Bacteriology by Holt *et al*. [20] were used for the identification of the isolates.

The fungi/moulds were identified by macroscopic and microscopic characteristics. The macroscopic properties was based on the visual characteristics of the growth on the plates, while the microscopic morphology was determined using the scheme of Pepper and Gerba[21] and Benson [19]. Lactophenol cotton blue stain was used to aid identification. The resultant morphology was compared using the guide provided by Pepper and Gerba (2005), Ellis *et al*. [22] and Benson [19].

2.4 Statistical Analysis

Statistical Package for Social Sciences software version was used for the statistical analysis of the log transformed microbial counts. Descriptive statistics i.e. mean and standard error values were expressed. A one-way analysis of variance was carried out at P = 0.05 and Duncan Multiple range test was used for mean separation.

3. Results and Discussion

Table 1 presents the microbial load of yellow mash cassava sold in major markets of Yenagoa metropolis, Bayelsa state, Nigeria The total microbial load ranged from 3.848 Log cfu/g in Agudama-Epie to 4.973 Log cfu/g in Akenfa (heterotrophic bacteria), 2.659 Log cfu/g in Akenfa to 3.793 Log cfu/g in Tombia (total coliform) and 3.371 Log cfu/g in Akenfa to 3.832 Log cfu/g in Swali (total fungi). *Salmonella-Shigella* were not detected in the yellow *gari* from the various markets from which the samples were obtained. However, there was no significance difference (P>0.05) among the markets for each of the microbial class.

Table 1: Microbial load of yellow *gari* sold in some major markets of Yenagoa metropolis, Nigeria

Markets	Total Heterotrophic Bacteria, Log cfu/g	Total coliform, Log cfu/g	Total Fungi, Log cfu/g	Salmonella-Shigella counts, Log cfu/g
Igbogene	4.918±0.532a	3.500±0.176ab	3.672±0.517a	ND
Akenfa	4.973±0.523a	2.659±0.305a	3.371±0.422a	ND
Agudama-Epie	3.848±0.535a	3.165±0.448ab	3.726±0.723a	ND
Tombia	3.962±0.464a	3.793±0.145b	3.724±0.454a	ND
Swali	3.954±0.463a	3.525±0.333ab	3.832±0.578a	ND

Each value is expressed as mean ± standard error (n = 3); the same letters along the column is not significantly different at P>0.05 according to Duncan Statistics; ND= Not Detected

The microbial load of white cassava flake sold in major markets of Yenagoa metropolis, Bayelsa state, Nigeria is presented in Table 2. The total microbial load ranged from 4.206 Log cfu/g in Tombia to 5.206 Log cfu/g in Swali (heterotrophic bacteria), 3.242 Log cfu/g in Tombia to 3.803 Log cfu/g in Swali (total coliform), and 3.887 Log cfu/g in Akenfa to 4.145 Log cfu/g in Swali (total fungi). Salmonella-Shigella counts were not detected in the white cassava mash from the various markets. However, there was no significance difference (P>0.05) among the markets for each of the microbial class.

Table 2: Microbial load of white *gari* sold in some major markets of Yenagoa metropolis, Nigeria

Markets	Total Heterotrophic Bacteria, Log cfu/ml	Total coliform, Log cfu/ml	Total Fungi, Log cfu/ml	Salmonella-Shigella counts, Log cfu/ml
Igbogene	4.223±0.582a	3.264±0.422a	3.908±0.245a	ND
Akenfa	5.049±0.474a	3.609±0.331a	3.887±0.257a	ND
Agudama-Epie	4.221±0.295a	3.694±0.187a	4.068±0.439a	ND
Tombia	4.206±0.235a	3.242±0.510a	4.090±0.155a	ND
Swali	5.206±0.393a	3.803±0.461a	4.145±0.371a	ND

Each value is expressed as mean ± standard error (n = 3); the same letters along the column is not significantly different at *P*>0.05 according to Duncan Statistics; ND = Not Detected

Based on the results of this study, It can be inferred that the susceptibility of the various *gari* to contamination by microbes is probably due to method of preparation and handling in the various market and is not significant (P>0.05). Most of the *gari* samples were within the acceptable limit for total aerobic bacteria and fungi counts, however, in few instances, it fell within the tolerable limits. However, ICMSF [23] cited in Olopade *et al*. [8], Izah *et al*. [24] reported the limits for total aerobic bacteria and fungi counts in the order ≤10^3 to be acceptable, 10^4 to 10^5 to be tolerable. According to the authors, coliform should not be found in ready to eat food hence allowable limit of 0.00.

The microbial density observed in this study have some similarity with the work of Olopade *et al*. [8], who reported that total aerobic plate count, total coliform and total fungi in the range of 2.0 x 10^2 to 1.1 x 10^4cfu/g, 0.00 to 7.1 x 10^3cfu/g and 0.00 to 6.0 x 10^2cfu/g respectively (white *Gari*), of 1.0 x 10^2 to 5.0 x 10^3cfu/g, 0.00 to 6.0 x 10^3cfu/g and 0.00 to 3.0 x 10^3cfu/g respectively (yellow *gari*).

Ijabadeniyi *et al*. [16] reported the microbial load of *Gari* and *lafun* from Ojo Oba Market, Akure, Nigeria as 14.3 x 10^4cfu/g, and 13.2 x 10^5cfu/g respectively (total viable aerobic bacteria counts), 1.6 x 10^4cfu/g, and 7 x 10^5cfu/g respectively (total fungal counts). Osho *et al*. [17] reported the average microbial population of *gari* from Ijebu-Igbo Central Market, Ogun State; Bodija Market, Ibadan, Oyo State and Garki Central Market, Abuja-Federal capita as territory 13.1 x 10^4cfu/g and 1.4 x 10^4cfu/g, for total viable bacterial counts and total fungi counts respectively. Obadina *et al*. [25] reported the fungi load of *gari* and *lafun* sold in some local markets in Abeokuta (Kuto, Osiele, Lafenwa, Iberekodo and Adatan), Ogun state as 1.79 x10^4cfu/g and 2.62 x 10^4cfu/g respectively.

Table 3 shows the microbial diversity found in each of the *gari* obtained from the different markets. The bacteria isolates include *Micrococcus* species, *Bacillus* species, *Pseudomonas aeruginosa*, *Staphylococcus aureus, Entrobacteraerogenes, E. coli, Klebsiella sp*, *Proteus* sp, *Corynebacterium* sp, while the fungi isolates are *Aspergillus niger, Fusarium* species, *Rhizopu s*sp, *Mucor* species and *Penicillium* species.

A wide array of microbes is found in the *gari* samples. However, the findings of this study is comparable to reported of Olopade *et al*. [8] who reported microbial diversity found in white and yellow *gari* sold in Ota, Ogun state as *Bacillus, Enterobacter, Pseudomonas, Staphylococcus* and *Klebsiella* spp. (bacteria), *Aspergillus niger, Aspergillus fumigatus, Fusarium, Rhizopus and Penicillium* spp. (Fungi). Obadina *et al*. [25] reported microbes found in some fermented indigenous food in Abeokuta (Kuto, Osiele, Lafenwa, Iberekodo and Adatan), Ogun state as *Aspergillus, Mucor, Rhizopus* and *Penicillium* spp. (*gari*) and *Aspergillus, Mucor, Rhizopus*spp. (*lafun*). But far from the reported of other authors. Adebayo-Oyetoro *et al*. [6] listed *Escherichia coli, Klebsiella oxytoca, Bacillus cereus, Staphylococcus aureus, and Clostridium sporogenes*as bacteria isolates which ferments cassava flour (*lafun*) in some locations in Ogun and Oyo States, Nigeria. Ijabadeniyi *et al*. [16] reported microbes associated with some indigenous fermented product such as *gari* to include *Pseudomonas, Bacteriodes, Actinomyces, Corynebacterium, Lactobacillus* spp. (bacteria), *Scolecotrichum graminis, Tallospora aspera, Passalora bacilligera, Varicosporium specie, Culicidosporagravida* and *Diplococcium spicatum* (mould), while *lafun* fermentation is associated with *Streptococcus, Lactobacillus a*nd *Listeria* spp. (bacteria), *Articulospora inflate, Aspergillus niger, Aspergillus rapens, Aspergillus flavus, Lemonniera aquatica*(mould). Osho *et al*. [17] reported microbes associated with fermented *Gari* to include *Leuconostoc, Pseudomonas, Bacteriodes, Actinomyces, Corynebacterium, Lactobacillus* spp. (bacteria), *Geotrichum candidum, Scolecotrichum graminis, Tallospora aspera, Passalora bacilligera, Culicidospora gravid, Diplococcium spicatum* and *Varicosporium* species(mould), while *lafun* fermentation is associated with *Streptococcus, Lactobacillus,* and *Listeria* spp. (bacteria), *Articulospora inflate, Aspergillus niger, Aspergillus rapens, Aspergillus flavus, Lemonniera aquatica*(mould).

Table 3: Microbial diversity of white and yellow *Gari* sold in some market in Yenagoa metropolis, Bayelsa state, Nigeria

Microbes	Igbogene	Akenfa	Agudama-Epie	Tombia	Swali
Bacillus sp	(+) [+]	(-) [+]	(+) [-]	(-) [+]	(-) [-]
Micrococcus sp	(-) [-]	(-) [+]	(-) [-]	(-) [-]	(+) [-]
Staphylococcus aureus	(+) [+]	(+) [+]	(+) [+]	(+) [+]	(+) [+]
Pesudomonas aeruginosa	(-) [-]	(+) [-]	(-) [+]	(-) [-]	(+) [-]
Enterobacter aerugenes	(+) [-]	(-) [-]	(+) [-]	(+) [+]	(-) [-]
E. coli	(+) [-]	(+) [+]	(+) [-]	(+) [+]	(-) [+]
Klebsiella sp	(-) [-]	(+) [-]	(-) [-]	(-) [-]	(-) [-]
Proteus sp	(-) [-]	(-) [-]	(+) [-]	(-) [-]	(-) [-]
Corynebacterium sp	(+) [+]	(-) [-]	(-) [+]	(+) [-]	(-) [-]
A. niger	(+) [+]	(+) [+]	(+) [+]	(+) [-]	(+) [+]
Mucor sp	(+) [+]	(-) [+]	(+) [+]	(+) [-]	(+) [+]
Fusarium sp	(-) [-]	(-) [-]	(+) [+]	(-) [-]	(-) [-]
Penicillin sp	(-) [-]	(-) [+]	(-) [-]	(+) [-]	(-) [-]
Rhizopus sp	(-) [-]	(+) [-]	(-) [+]	(-) [-]	(+) [-]

-= absence; + = present; ( ) = white *gari*; [ ] = yellow *gari*

Note: some of the microbial isolates only occurred in one of the three replicate in each market

Therefore, the occurrence of coliforms in the *gari* indicates that their quality is poor. Coliforms and other microbes have invaded the samples during cooling processes after frying/sieving and sales. Most of the species of microbes found in the *gari* samples are pathogenic and are associated with environmental contaminations. The presence of *Escherichia coli* and *Enterobacter aerogenes*is an indication of Coliforms which may have occurred during handling due to poor hygiene including the use of unsterile basin for display of *gari* prior to sales. *Staphylococcus aureus* is found in the nasal passage, hands and skin of humans as normal flora [26] and may have entered the *Gari* during handling processes.

Moulds found in the *gari* are primarily due to environmental contaminants probably due to their ability to produce spores [8]. They have been implicated in ready to eat foods and in unregulated or spontaneous fermentation [8, 26]. However, most of the fungi isolated from the *gari* are pathogenic. *Penicillium, Fusarium* and *Aspergillus*

species identified in this study are known to produce toxins. For instance, *Aspergillus* species produces aflatoxins and ochratoxins; *Fusarium* species produces Moniliformin and Fumornisins; and *Penicillium* species produces Citrinin and Cyclopiazonic acid [27]. Ijabadeniyi *et al*. [16] stated that *A. flavus* produces aflatoxin which is carcinogenic. According to Izah *et al*. [26], these fungi are opportunistic systemic mycoses that cause diseases in immunocompromized patients. Some of the disease caused conditions are Aspergillosis (caused by some species of Aspergillus), mucormycosis (caused by some species of Mucor), hyalohyphamycosis (caused by some species of *Penicillium* and *Fusarium*)[26].

4. Conclusion

Fermented cassava mash popularly known as *gari* is a staple food to several million to people not only in Nigeria but across several West African countries. Cassava is typically used for the production of several products including food when processed into *gari*. *Gari* is consumed raw and used for the production of *Eba* a popular food made from *gari* with addition of boiled water. This study investigated the microbiological quality of both yellow and yellow *gari* sold in five markets in Yenagoa metropolis, Nigeria. The study found that microbial counts (total aerobic bacteria and fungi is within acceptable and tolerable level). Also, Coliforms were found in the *gari* which naturally should not be present in ready to eat food such as *gari*. Generally, the presence of some of these microbes in both *gari* types is an indication of contamination. Which probably have occurred during handling, preservation and selling point. The occurrence of these microbes could be reduced through improved hygienic condition of the processors, sellers including the storage and selling materials such as bowl/basin.

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