



Microbiological Assessment of Cassava Based Foods (Garri And Akpu) Within Abakaliki Metropolis

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ABSTRACT

Garri and Akpu is an African traditional fermented food product prepared from cassava that is widely accepted by both rural and urban dwelling peoples with little concern about those microorganisms that are associated with it. The present study was undertaken to investigate the microorganisms associated with cassava based product (Garri and Akpu). A total of 400 samples (50 white Garri, 50 yellow Garri and 100 Akpu) were purchased within Abakaliki Metropolis, Ebonyi State. Using serial dilution method, the samples were plated on different microbiological media. Organisms growing on the media were purified and identified using microbiological standards. It was found out that all the tested samples were positive for microbial contamination. Out of 400 cassava based foods (50 white Garri, 50 yellow Garri and 100 Akpu) analyzed, a total of 104 bacteria isolates were obtained. Out of which 44 bacteria isolates (11 Salmonella species, 8 Citrobacter species, 7 Serratia species, 7 Shigella species, 8 Klebsiella species and 3 Proteus species) were from White Garri; 37 bacteria from Yellow Garri (5 Citrobacter species, 3 Salmonella species, 7 Shigella species, 5 Klebsiella species, 8 Proteus species, 9 Serratia species) and 23 bacterial isolates (14 Escherichia species and 9 Staphylococcus species) from Akpu. A total of 48 fungal isolates were obtained from the 400 cassava based foods analyzed. 17 fungal isolates (6 Mucor species, 5 Aspergillus species, 4 Rhizopus species and 2 Fusarium species) were obtained from White Garri, 13 fungal isolates (5 Rhizopus species, 4 Aspergillus species, 3 Fusarium species and 1 Mucor species) from Yellow Garri and 18 fungal isolates (6 Aspergillus species, 6 Fusarium species, 4 Rhizopus species and 3 Mucor species) where from Akpu. This present study revealed high bioload and vast array of microorganisms in market Garri and Akpu. These are threatening and alarming and suggest early warning signals indicating the level of safety of available market Garri and Akpu. Hence adequate sanitation practice should be enforced concerning the sale of cassava based foods. Personal hygiene of hawkers and sanitation of utensils are important.

Keywords: Bacteria, fungi, Akpu, Garri, hygiene, Abakaliki Metropolis

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop in Africa, Asia, South America and India where it provides over 50% of the average daily intake in some countries (Oyewole and

Ogundele, 2001), cassava is also an important food in the tropics, it has two major deficiencies which are carried over into those foods made from it: Its content of toxic cyanogenic glucosides and its low content of protein and amino acids (Okafor *et al.*, 1999).

The Fufu also called “Akpu” in Ibo is a meal of soaked fermented cassava roots widely consumed in particularly in the southeastern parts of the country (Iwuoha and Eke, 1996; Sobowale *al.*, 2007).

Garri is a popular food in Nigeria derived from the fermentation of mash obtained from the enlarged root of the cassava plant, *Manihot esculenta*, Crantz (Okafor *et al.*, 1999).

Several workers have studied the microbiology of cassava fermentation for Fufu “Akpu” production (Okafor *et al.*, 1984; Oyewole, 1991; Brauman *et al.*, 1996; Sobowale *et al.*, 2007). These previous works have concentrated on the microorganisms associated with cassava fermentation for Fufu “Akpu” production with the aim of optimizing the microbial processes and improving the organoleptic appeal of the food. However, much has not been done on the aspect of microbial quality of Fufu “Akpu” and Garri from the public health point of view. Hence this work was aimed to ascertain the microorganisms associated with cassava based foods (Akpu and Garri) within the Abakaliki Metropolis, Ebonyi State, Nigeria.

MATERIALS AND METHODS

Study Area

This study was conducted within 10 markets in Abakaliki Metropolis, Ebonyi state. The study area is located between Latitude 06⁰ 4'N and longitude 08⁰ 5'E and rainfall pattern is bimodal (April-July), September-November with a short spell sometimes in August. The annual rainfall is between 1000mm-1500mm. The vegetation of the area is predominantly derived Savannah. The mean annual temperature is about 24°C and the relative humidity is between 60-80% (Ofomata, 1975).

Sample Collection and Preparation

A total of 400 samples (50 white Garri, 50 yellow Garri and 100 Akpu) were purchased between May-October, 2013. Samples were collected at regular intervals evenly spread over the study period. These samples were collected randomly from 10 selected markets within Abakaliki Metropolis, Ebonyi State, Nigeria.

Sample Preparation

The samples were prepared using the method described by Thoha *et al.* (2012) with slight modification. 10g of each sample was added to 90ml of 0.1% (w/v) peptone water and homogenized by rolling between the palms at medium speed. Serial decimal 10 fold dilution was prepared by transfer of one millimeter of initial suspension into a tube containing 9ml of 0.1% (w/v) peptone water. These operations were repeated using a new sterile pipette to obtain 10⁻² through 10⁻¹⁰ dilutions.

Isolation and Identification of Bacteria and Fungi

The suspensions of the samples prepared above were inoculated onto nutrient agar, MacConkey agar and potato dextrose agar. The plates were incubated at 27°C for 72 hours after which slide cultures were prepared for microscopic examination and biochemical identification (Cheesbrough, 2006).

Subsequently, Shigella Salmonella Agar (SSA) was also used for the isolation of *Shigella* and *Salmonella* species.

RESULT AND DISCUSSION

The result of the microbial examination of the cassava based foods (Garri and Akpu) showed that 8 bacteria and 4 fungi isolates were isolated from this work. These bacterial isolates from Garri

encompass *Citrobacter* species, *Salmonella* species, *Shigella* species, *Klebsiella* species, *Proteus* species and *Serratia* species and from Akpu include *Staphylococcus* species and *Escherichia* species as shown in Table 1, while the fungal isolates were *Aspergillus* species, *Rhizopus* species, *Mucor* species and *Fusarium* species from both Garri and Akpu as shown in Table 2.

Table 1: Characteristic of bacteria isolates isolated from cassava based foods (Garri and Akpu)

Morphological characterization		Gram staining	Citrate Test	Oxidase Test	Indole Test	Voges Proskauer	Motility Test	Sugar Fermentation Test			Suspected Organisms
Colour	Consistency /Texture							Glucose	Lactose	Fructose	
Colourless	Small round colonies	-	+	-	-	-	+	+	+	+	<i>Citrobacter</i> spp.
Milky	Small round colonies	-	+	-	-	-	+	+	-	-	<i>Salmonella</i> spp.
Light yellow	Raised /smooth edge	-	-	-	-	-	-	+	-	+	<i>Shigella</i> spp.
Pink	Mucoid colonies	-	+	-	-	+	-	+	+	+	<i>Klebsiella</i> spp.
Bluish green	Small round colonies	-	+	-	-	-	+	+	-	+	<i>Proteus</i> spp.
Dark red	Small round colonies	-	-	-	-	+	+	+	+	-	<i>Serratia</i> spp.
Greenish	Rough surface	-	+	-	+	-	-	+	+	-	<i>Escherichia</i> spp.
Creamy	Raised/ smooth edge	+	+	-	-	-	-	+	-	-	<i>Staphylococcus</i> spp.

Table2: Morphological and Microscopic of fungi isolated from cassava based foods (Garri and Akpu)

Morphological Characteristics	Microscopic Examination	Suspected Organisms
Velvety filamentous white growth that sporulate black powdery spores	Long septate hyphae with conidiophore bearing brown spores and phialide at its apex	<i>Aspergillus</i> species
Long hyphael growth which	Non-septate, branched mycellium with	<i>Rhizopus</i> species

sporulated within two days to turn to black spore	round shaped sporangia	
White and wooly aerial growth that darkens as it sporulate	Non-septate hyohae with straight sporangiophore with many spherical spores.	<i>Mucor</i> species
Pink fluffy and spreading colonies which is creamy around edges	Septate hyphae with sickle chlamyospores at the hyphae	<i>Fusarium</i> species

Out of 400 cassava based foods (50 white Garri, 50 yellow Garri and 100 Akpu) analyzed, a total of 104 bacteria isolates were obtained. Out of which 44 bacteria isolates (11 *Salmonella* species, 8 *Citrobacter* species, 7 *Serratia* species, 7 *Shigella* species, 8 *Klebsiella* species and 3 *Proteus* species) were from White Garri; 37 bacteria isolates (9 *Serratia* species, 8 *Proteus* species, 7 *Shigella* species from Yellow Garri, 5 *Citrobacter* species, 5 *Klebsiella* species and 3 *Salmonella* species) and 23 bacterial isolates (14 *Escherichia* species and 9 *Staphylococcus* species) from Akpu (Table 3).

Table 3: Percentage frequency of bacterial isolates from cassava based foods (Garri and Akpu)

Bacterial Isolates	White Garri	Yellow Garri	Akpu
<i>Citrobacter</i> species	8 (18.2%)	5 (13.5%)	0
<i>Salmonella</i> species	11 (25.0%)	3 (8.1%)	0
<i>Shigella</i> species	7 (15.9%)	7 (18.9%)	0
<i>Klebsiella</i> species	8 (18.2%)	5 (13.6%)	0
<i>Proteus</i> species	3 (6.8%)	8 (21.6%)	0
<i>Serratia</i> species	7 (15.9%)	9 (24.3%)	0
<i>Escherichia</i> species	0 (0.0%)	0 (0.0%)	14 (60.9%)
<i>Staphylococcus</i> species	0 (0.0%)	0 (0.0%)	9 (39.1%)
Total	44	37	23

A total of 48 fungal isolates were obtained from the 400 cassava based foods (50 white Garri, 50 yellow Garri and 100 Akpu) analyzed. 17 fungal isolates (6 *Mucor* species, 5 *Aspergillus* species, 4 *Rhizopus* species and 2 *Fusarium* species) were obtained from White Garri, 13 fungal isolates (5 *Rhizopus* species, 4 *Aspergillus* species, 3 *Fusarium* species and 1 *Mucor* species) from Yellow Garri and 18 fungal isolates (6 *Aspergillus* species, 6 *Fusarium* species, 4 *Rhizopus* species and 3 *Mucor* species) where from Akpu (Table 4).

Table 4: Percentage frequency of fungi isolates from cassava based foods (Garri and Akpu)

Bacterial Isolates	White Garri	Yellow Garri	Akpu
<i>Aspergillus</i> species	5 (29.4%)	4 (30.8%)	6 (33.3%)
<i>Rhizopus</i> species	4 (23.5%)	5 (38.5)	4 (22.2%)
<i>Mucor</i> species	6 (35.3%)	1 (7.7%)	3 (16.7%)
<i>Fusarium</i> species	2 (11.8%)	3 (23.0%)	5 (27.8%)
Total	17	13	18

Cassava is a plant that has been processed into many products and there are still emerging new products from cassava. These products have fed millions of people and others have economic value. This has elicited interest in cassava-based products (Ukwuru and Egbonu, 2013). In Nigeria, cassava is processed into gari, fufu, pellets for compounding animal feeds and kpokpo gari (Lancaster *et al.*, 1982 and Tewe, 1986) and also into instant aromatized (fermented) flour (Okpokiri *et al.*, 1984).

In Nigeria, the sale and distribution of garri in local markets is associated with practices such as display of product in open buckets, bowls and mats at points of sale and the use of bare hands during handling and sales. These unhygienic practices, which may lead to microbial contamination due to deposition of bioaerosols on exposed products (Ogugbue *et al.*, 2011). Previous reports have revealed high bioload and a vast array of microorganisms in market samples of garri and other cassava based products (Ijabadeniyi, 2007; Ogiehor *et al.*, 2007; Omafuvbe *et al.*, 2007; Amadi and Adebola, 2008; Ogugbue *et al.*, 2011 and Odom *et al.*, 2012).

Out of 400 cassava based foods (50 white Garri, 50 yellow Garri and 100 Akpu) analyzed, a total of 104 bacteria isolates were obtained. Out of which 44 bacteria isolates (11 *Salmonella* species, 8 *Citrobacter* species, 7 *Serratia* species, 7 *Shigella* species, 8 *Klebsiella* species and 3 *Proteus* species) were from White Garri; 37 bacteria isolates (9 *Serratia* species, 8 *Proteus* species, 7 *Shigella* species from Yellow Garri, 5 *Citrobacter* species, 5 *Klebsiella* species and 3 *Salmonella* species) and 23 bacterial isolates (14 *Escherichia* species and 9 *Staphylococcus* species) from Akpu (Table 1, 3).

Salmonella species, *Citrobacter* species, *Serratia* species, *Shigella* species, *Klebsiella* species and *Proteus* species were isolated from White Garri and Yellow Garri (Table 1). *Salmonella* species (25.0%) were the highest bacteria isolated from White garri, followed by *Citrobacter* species (18.2%) and *Klebsiella* species (18.2%), *Shigella* species (15.9%), *Serratia* species (15.9%), while *Proteus* species (6.8%) recorded the least. *Serratia* species (24.3%) were the highest bacteria isolated from Yellow Garri, followed by *Proteus* species (21.6%), *Shigella* species (18.9%), *Klebsiella* species (13.6%), *Citrobacter* species (13.5), while *Salmonella* species (8.1%) recorded the least (Tabl 3). It also observed that there is more microbial contamination in White Garri than Yellow Garri. This may be due to the antimicrobial property of the oil added to it. The result of this work is contrary to the work of Thoha *et al.* (2012), who reported more microbial contamination in Yello Garri than in White Garri.

The result of this work is in line with the work of Ogugbue *et al.* (2011) who isolated *Salmonella* species from post processed garri on sale in Port Harcourt Markets. *Salmonella* causes food poisoning and typhoid fever (Ekperigin and Nagaraja, 1998 and Parry *et al.*, 2002) and are particularly effective at causing human infections because they can survive a series of harsh conditions which include strong acids in the stomach (Oguegbu *et al.*, 2011). The presence of *Shigella* species as shown in this work is likely to be characterized by diarrhea and vomiting (Singleton, 1995; Frazier and Westhorf, 2004; Eze *et al.*, 2008). Ogiehor *et al.* (2007) isolated *Klebsiella* species from garri sold in some selected states of Southern Nigeria, which is in line with the result of this study. *Klebsiella* organisms can lead to a wide range of disease states, notably pneumonia, urinary tract infections, septicemia, and soft tissue infections (Podschun and Ullmann, 1998). *Proteus vulgaris* has been reported to be isolated from dried cassava powder (garri) in Ogun State (Thoha, 2012). This is in agreement with the result obtained in this work and also an indication that *Proteus* species can be isolated from garri.

Serratia species and *Citrobacter* species isolated in this study, is contrary to most work on the bacteriological analysis of Garri (Ogugbue *et al.* (2011) Ijabadeniyi, 2007; Omafuvbe *et al.*, 2007; Ogiehor *et al.*, 2007; Omafuvbe *et al.*, 2007; Amadi and Adebola, 2008; Ogugbue *et al.*, 2011; Thoha, 2012 and Odom *et al.*, 2012). Akpomie *et al.* (2012) reported the presence of *Serratia marcescens* from cassava root peels, this is also an indication that *Serratia* species can be isolated

from Garri, which is a cassava based food. These organisms (*Serratia* species and *Citrobacter* species) are capable of causing urinary tract infection.

A total of 48 fungal isolates were obtained from the 400 cassava based foods (50 white Garri, 50 yellow Garri and 100 Akpu) analyzed. 17 fungal isolates (6 *Mucor* species, 5 *Aspergillus* species, 4 *Rhizopus* species and 2 *Fusarium* species) were obtained from White Garri, 13 fungal isolates (5 *Rhizopus* species, 4 *Aspergillus* species, 3 *Fusarium* species and 1 *Mucor* species) from Yellow Garri and 18 fungal isolates (6 *Aspergillus* species, 6 *Fusarium* species, 4 *Rhizopus* species and 3 *Mucor* species) where from Akpu (Table 2, 4).

Mucor species (35.3%) were the highest fungi isolated from White Garri, followed by *Aspergillus* species (29.4%), *Rhizopus* species (23.5%) and *Fusarium* species (11.8%) recorded the least. *Aspergillus* species (33.3%) were the highest fungi isolated in Yellow Garri, followed by *Fusarium* species (27.8%), *Rhizopus* species (22.2%), while *Mucor* species (16.7%) were the least isolated (Table 4). The result of this study is in line with the work of Thoha *et al.* (2012), who isolated *Aspergillus niger* and *Aspergillus flavus* from both White Garri and Yellow Garri in Ogun State. The frequency of occurrence of the isolated fungi were compared for both Yellow Garri and White Garri and it was observed that the frequency of occurrence of *Aspergillus* species were higher in White Garri than Yellow Garri. This result is in conformity with the work of Thoha *et al.* (2012). Oguegbu *et al.* (2011) also reported the presence of *Aspergillus* species, *Fusarium* species and *Mucor* species among other fungi in post processed garri sold in Port Harcourt Market. The result of this study therefore showed that most fungi associated with the deterioration of Garri samples are xerophilic moulds such as *Aspergillus* species and *Fusarium* species. It is henceforth important to develop a strategy to antagonize their growth and survival in this commodity in order to neutralize the potential of these organisms serving as agents of food borne diseases.

Akpu is an important item of diet of a high proportion of Nigerian population, particularly in the eastern part of the country. The production of *fufu* from cassava tubers depend on the action of microorganisms, some of which originate from the raw materials and others from the atmosphere and from the microbiota of the human processors (Ekundayoa and Okoroafor, 2012). Since there is no deliberate effort on the part of the local processors to control the types or the numbers of microorganisms in the production process, the organoleptic properties, flavor and the overall microbiological quality and safety of the product can vary considerably from locality to locality (Sobowale *et al.*, 2007 and Padonou *et al.*, 2009). The overall quality of the product will be determined by the types of microorganisms introduced into the retting cassava tubers from the raw materials, water, processors and the environment, the microbial interactions and the resulting physicochemical interactions which eventually regulate the number and types of the microorganisms that survive to the end of the process (Brauman *et al.*, 1996; Padonou *et al.*, 2009). The result of this study is in line with the work of Omafuvbe *et al.* (2007), who revealed the presence of *Staphylococcus* species and *Enterobacter* species from Lafun-fermented cassava products sold in Ile-Ife. Subsequently, Ekundayoa and Okoroafor (2012) revealed the presence of *Staphylococcus aureus* and *Escherichia coli* in Fufu in some communities around Umudike. The presence of *Staphylococcus* species in Akpu sold in Abakaliki Metropolis could be as a result of contamination of the fermentation system from the human processors or from the environment. Adegoke and Babalola (1988) reported that the presence of *Staphylococcus* species has been associated with fermenting foods of plant origin especially vegetable products. Desse and Taye (2001) had noted that *Staphylococcus* species may survive the cassava fermentation process. Since some strains can produce potent exotoxins that are capable of causing food intoxication, the presence of *Staphylococcus* species in cassava retting implies some public health importance and requires further study (Ekundayoa and Okoroafor, 2012). The presence of *Escherichia* species in food samples indicate fecal contamination which could be attributed to unhygienic nature of the food handlers and food preparation areas as recorded (Abdullahi, 2004 and Edema *et al.*, 2005).

Aspergillus species (33.3%) was the highest fungi isolated from Akpu, followed by *Fusarium* species (27.8%), *Rhizopus* species (22.2%) and *Mucor* species (16.7%) the least. In conformity to the result of this work, Ekundayoa and Okoroafor (2012) revealed the presence of *Aspergillus* species, *Candida* species, *Penicillium* species and *Saccharomyces* species in Fufu (Akpu in some communities around Umudike). *Aspergillus* spp in the food may lead to food poisoning, since many of these fungi are toxin producing organisms. Ubiquitous in the environment and originated from the market display areas (Odom *et al.*, 2012).

CONCLUSION

This present work revealed high bioload and vast array of microorganisms in market Garri and Akpu. These are threatening and alarming and suggest early warning signals indicating the level of safety of available market Garri and Akpu. It also warrants renewed vigilance on the efficacies of food processing conditions, handling techniques and handlers technical knowhow, hygiene practices and safety of finished products. In addition, strict application and implementation of quality control, quality assurance, good manufacturing practice and the hazard analysis critical control point principles will help to ensure the safety of Garri and Akpu consumed by several people in Abakaliki Metropolis and Africa in general.

The high array and bioload microorganisms recorded may be associated with inadequate post processing handling practices such as spreading on the floor, mat and sometimes on high density polyethylene spread on the floor after frying to allow it to cool before sieving into finer grains; and the open display in bowls and basins in the market, measurement with the aids of bare hands, coughing and sneezing while selling and the use of non microbiologically determined hessian bags for packaging and haulage.

Adequate sanitation practice should be enforced concerning the sale of cassava based foods. Personal hygiene of hawkers and sanitation of utensils are important. Hawkers should be enlightened on hygienic practices.

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