MICROBIOLOGICAL ANALYSIS OF COMMERCIALLY SOLD GARI IN EKITI STATE

BY

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BEING A PROJECT WRITE UP

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CERTIFICATION

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DEDICATION

I dedicate this work to the God almighty whose boundless infinite love, goodness, guidance, favour, gave me a sense of knowledge and wisdom to successfully complete this project work.

Also to my supervisor, beloved parents Mr & Mrs Anyaogu and my wonderful sister Chioma Dickson.

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My achievement in life has been made possible by God and to Him be all the glory. I also express my sincere gratitude to others who contributed to my achievement.

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To you all, I say God bless.

ABSTRACT

The microbiological assessment of gari sold in three different markets (Oye, Ayede and Ilupeju) in Oye Ekiti LGA Nigeria was carried out. A total of twenty five (25) samples were studied. Nutrient agar, MacConkey agar were used for total heterotrophic count (THPC)), Coliform count (CC) and Potato-Dextrose agar plates for Fungal count respectively. The pH and moisture content of the samples were also determined using the appropriate method. Antimicrobial test was also carried out on the bacteria isolates to determine their susceptibility to a given antibiotics. The total heterotrophic count (THPC) of white gari and yellow gari ranged from 2×10^4–5×10^4CFU/g and 2×10^4– 7×10^4 CFU/g) respectively while fungi count of white and yellow gari samples ranged from 2×10^2 –8×10^2CFU/g and 1×10^2–7×10^2 CFU/g respectively. There was no presence of coliform in all the samples. pH values of white and yellow samples ranged from 5.90– 6.80 and 6.09 – 6.82 while the moisture content of white and yellow gari ranged from 1.00 – 1.42 and 1.08 – 1.58 respectively. A total of seven bacterial genera isolates and two fungal genera were obtained, out of which were *Bacillus polymyxa, Bacillus licheniformis, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis, Staphylococcus aureus and Bacillus marcerans, Aspergillus niger, Aspergillus fumiatus, Aspergillus flavus and penicillium spp.* The zone of inhibition of different bacteria isolates ranges from 10–35mm. The highest zone of inhibition was observed in plate of *Bacillus subtilis* with Augumentin(AU) 30ug which shows zone of inhibition of 35mm, while the lowest zone of inhibition was observed in plate of *Bacillus cereus, Staph. aureus, Salmonella typhi* with Amoxacillin(AM) 30ug with zone of inhibition 10mm respectively. It is henceforth important to develop a strategy to properly package and store this product to reduce microbial contamination.

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1.0 INTRODUCTION

1.1 BACKGROUND OF STUDY

1.1.2 Introduction to gari

Gari is a dehydrated coarse product obtained from peeled, grated, fermented and roasted cassava tuber (Adejumo *et al.,* 2015). It is a popular and common cassava staple consumed by several millions of people across various ethnic and socio-economic classes in the West Africa Sub region (Jekanyinfa and Olajide, 2007; Ogiehor *et al.,* 2005).

Gari is rich in starch, fibre and contains some essential vitamins (Adepoju *et al.,* 2010; Oboh *et al.,* 2002; Okwu and Awurum, 2001). Its high fibre content helps in preventing or at least reducing the likelihood of constipation and bowl diseases (Adepoju *et al.,* 2010). It is classified based on texture, length of fermentation, region or place where it is produced and colour imparted by the addition/non addition of palm oil. It has a high swelling capability and can absorb up to four times volume of water (Jekayinfa and Olajide, 2007).

The process of preparation of cassava into gari involves grating and fermentation of the tuber (Amadi and Adebola, 2008). The cassava pulp is put in jute bags and weighed down with hydraulic press to dehydrate for 2-5 days. The fermented lump is subsequently fried at high temperature in a pan (Amadi and Adebola, 2008).

The quality of the product depends on the management of each stage of processing and handling (Adejumo *et al.,* 2015). The practices associated with the production, processing and handling such as drying on the floor, mat, rock, road side etc after frying and display in the open bowls, bags and mat at point of sale increases solid and microbial contamination (Ogiehor and Ikenehomeh, 2012). The processing of cassava into gari usually takes three to five days both at household and factory levels and its average moisture content is about 8-14 percent (Adejumo *et al.,* 2005).

Gari is taken in various forms in Nigeria, which include viscous paste (eba), soaking in cold water, mixed with other food like beans, moin-moin, etc. Gari it self may not constitute health hazard, since it has been estimated that various operation involved during processing usually resulted in reduction in total cyanide content (Adejumo *et al.,* 2015). The unhygienic handling and poor sanitary measures that are very obvious as being observed between the last stage of production and the time its being displayed in the markets where the main patronage of many consumers could constitute serious health implications as many chances have been

given to contamination by organisms of epidemiological importance (Arasi and Adebayo, 2000).

The contamination of gari by certain species of microbes which happens due to the way the food is been handled from processing to consumption stage and their associated cause of food borne illnesses and public health threat, calls for attention and regular surveillance for their presence in foods. It is in line with the foregoing, that this study was designed to evaluate the microbial Load of commercially sold gari and to make necessary suggestions for proper handling.

2.1 Aim of the study

This study has been undertaken:

To evaluate the microbial Load of gari purchased from three different markets (Oye, Ilupeju and Ayede) in Oye Local Government Area in Ekiti state.

2.1.1 **Objectives of the study**

a) To isolate the organism present in the gari samples.
b) To identify the organism using their morphological characteristics on growth media.
c) To carry out antimicrobial/Antibiotics susceptibility test on the isolated organisms.

CHAPTER TWO

2.0 LITTERATURE REVIEW

2.1 What Is Gari

Gari is a fermented dry product from cassava. It is a cheap and popular meal consumed by mixing with boiling water to form paste and eaten with soup in many rural areas of Nigeria especially among the low income earners (Makanjuola *et al.*, 2012).

Arisa *et al.* (2011), reported the use of gari as snack. It could be soaked with cold water and eaten with groundnut, fried fish, coconut with addition of sugar, palm kernel, groundnut cake (kwuli kwuli), and fermented maize snacks kokoro. Beverages and milk may also be added as complements. Eba is another food prepared from gari. The granules are added into hot water and stirred to form a stiff paste which can be eaten with indigenous soups or stew (Asegbeloyin and Onyimonyi, 2007).

Gari is a staple food and therefore consists of majorly carbohydrate. The protein content of cassava is low and of poor quality (Oluwamukomi and Jolayemi, 2012), due to this a lot of studies had been conducted to improve the nutritional value of gari using soybean, melon, groundnut and sesame seed flour (Osho, 2003; Oluwamukomi *et al.*, 2005; Oluwamukomi and Jolayemi, 2012; Arisa *et al.*, 2011; Oluwamukomi, 2015). Melon is a cucurbit crop that belongs to the Cucurbitaceae family with protein content ranging from 33.80-39.96% (Abiodun and Adeleke, 2010).

2.2 How Is Gari Produced

Gari production is a tasking and burdensome procedure and its method of production differs from one locality to another (Olopade *et al.,* 2014). In a typical production of Gari, the cassava tubers are peeled, washed, grated and packed into closely woven bags. The poisonous juice can then be removed by placing a heavy object on the bag and the contents of the bag are allowed to undergo spontaneous solid state fermentation for several days at ambient temperatures (Ray and Sivakumar, 2009). According to Akindahunsi *et al.*,(1999); Azam- Ali *et al.* (2003) the grated tubers are allowed to ferment in order to preserve the product, reduce cyanide and enhance its flavour.

Osho and Dashiell (2002) stated that frying at high temperatures dries the fermented pulp to about 10% moisture content and may result in partial dextrinization of starch. Asegbeloyin

and Onyimonyi, (2007); Harbor and Ogundu(2009) also revealed that frying destroys enzymes and microorganisms and aids in eliminating cyanide gas from the product.

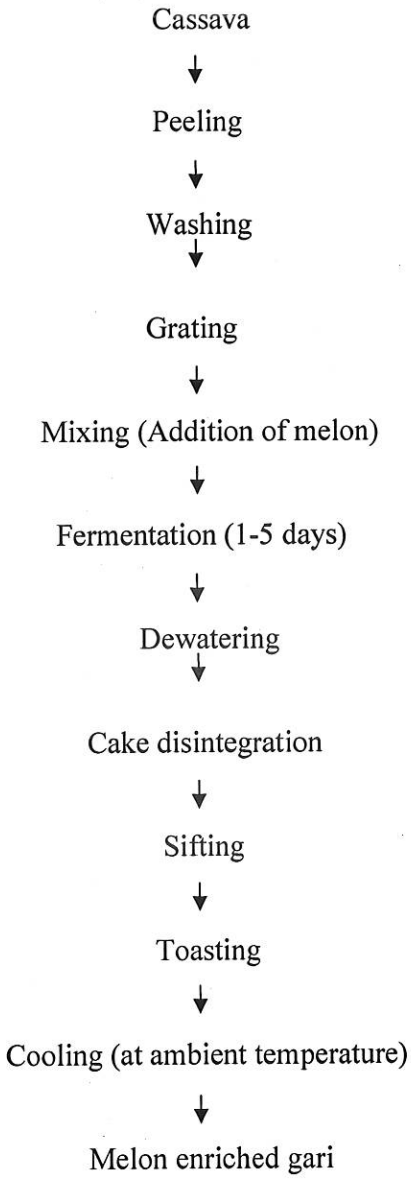

Figure 1: Production of gari enriched with melon flour

Source; (Abiodun *et al.*, 2016)

2.3 Types Of Gari

Gari is classified/grouped based on texture, length of fermentation, region or place where it is produced and colour imparted by the addition/non addition of palm oil Olopade *et al.*, (2014).

2.3.1 Red Gari

This is the type of gari commonly found in the Mid – Western part of Nigeria. It is also called Bendel gari. It is produced following harvesting of cassava, peeling, grating, dewatering, fermenting, sieving, frying and bagging. Red palm oil is added after grating the cassava and the gari is allowed to ferment for two to three days also adding of palm oil to the gari further helps to reduce the cyanide content and gives it a unique flavour. Yellow gari is preferred and can cost twice as much, making it less available to poorer households (Afolabi, 2009).

2.3.2 White Gari

Same as Bendel gari, left to ferment for two to three days as well, but red palm oil is not added during processing. It is produced following harvesting of cassava, peeling, grating, dewatering, fermentation (optional), sieving, frying and bagging (Afolabi, 2009).

2.3.3 Ijebu Gari

Ijebu gari is made same way too, but allowed to ferment for up to seven days. No palm oil is added. It is also fried to become much crisped. It characteristically has a very sharp tasted and less starchy. Many people from the Western part of Nigeria love this and find it great for "soaking" Olopade *et al.*, (2014).

2.4 Health Benefits and Effects of Gari on the body

According to Nyerhovwo (2004), among the starchy staples foods, cassava products such as gari gives a carbohydrate production which is about 40% higher than rice and 25% more than maize with result that cassava is the cheapest source of calories for both human nutrition and animal feeding.

Apart from being a rich source of energy owing to its carbohydrate content, gari is also a good source of fibre which help promote bowel emptying and prevent stomach cancer (Adepoju *et al.,* 2010). The main side effect of gari consumption is related to the cyanide content of cassava from which gari is produced, Increased intake of cyanide in the diets of man can lead or contribute to goiter, cretinism, paralysis and neurological disorders (Ojo and Akande, 2013).

Gari is made from cassava which is known to contain hydrocyanic acid. Although the processing of gari significantly reduces its Cyanide content, it has been linked to eye defects which is the main disadvantage of gari consumption (Ahaotu *et al.,* 2013).

2.5 **Microorganism involved in gari fermentation**

One of the most popular foods derived from fermented cassava is gari (Yao *et al.,* 2009). It is consumed either soaked in cold water or stirred in boiling water to make a stiff paste and consumed with choice soup (Guira *et al.,* 2016). Gari can be yellow (if fortified with red palm oil) or white, although "gari" from bio-fortified cassava is gaining popularity now.

Seventy percent of cassava processed as human food is "gari" (Moslehi-Jenabian *et al.*, 2010). Its wide consumption is attributed to its relatively long shelf life and its easy preparation as a meal. There are variations in the gari produced within the sub-region in terms of physical, chemical and sensory qualities which also emphasizes precautions on unit operations that have implications on finished product quality and safety (Adeniran and Ajifolokun, 2015).

It is commercially prepared by several forms of consortia of microorganisms (Behera1 and Ray, 2016). Cassava fermentation is mainly due to lactic acid bacteria (LAB), *Lactobacillus* sp. and to a lesser extent *Streptococcus* sp. is responsible for acid production and gari flavour (Behera and Ray, 2016).

(Oguntoyinbo and Dodd, 2010) investigated the microbial dynamics and diversity during solid state fermentation of cassava during gari production in West Africa. Several advance molecular techniques, such as 16S rDNA gene sequence analysis, pulsed field gel electrophoresis (PFGF) analysis were used to monitor the bacterial dynamics during cassava fermentation. The lactic acid bacterial species and their close relatives, including *Lactobacillus plantarum*, *Lactoacillus fermentum*, *Lactobacillus. pentosus*, *Lactobacillus. acidophilus* and *Lactobacillus. casei* were identified.

Oyewole and Odunfa (2008) showed that *L. plantarum* is a common occurrence in fermenting cassava. *L. plantarum* has been shown previously to be the predominant LAB species in sour cassava starch (Oyewole and Odunfa 2008). The majority of LAB strains play major role in pH reducton and is important to reduce the levels of contaminating microorganisms present on the raw materials, utensils and the environment which can compete with the starters for nutrients (Holzapfel, 2002).

Also according to Ahaotu *et al.,* (2013) on study of Fermentation of undewatered cassava pulp by linamarase producing microorganisms and effect on nutritional composition and residual cyanide using Cassava waste water (whey) collected from a small scale gari processing factory in Benin City. It was reported that the following bacteria isolates were isolated from waste water collected from the cassava processing factory; *Alcaligenes faecalis*, *Lactobacillus plantarum*, and *Leuconostoc cremoris* while *Geotrichum candidum* was isolated for yeast. The various bacterial isolates obtained from the cassava waste water have also been isolated from fermenting cassava by various workers (Amao-Awua *et al.*, 1996; Essers *et al.*, 1995; Kobawala *et al.*, 2005). It was reported that LAB and yeasts are believed to be the major fermenting microorganism. However, the genera of microorganisms involved in cassava fermentation for gari production would depend on some factors such as how the cassava was processed before fermentation and probably their ability to detoxify linamarin, and the amount of cyanide produced from linamarin breakdown (Ejiofor and Okafor, 2008). It will also depend on the source of the cassava, the types and numbers of initial microorganisms on the pulp, and the processing environment (Ahaotu *et al.,* 2013). The microorganisms isolated and used for the study were shown to have the ability to produce linamarase which have been reported by some workers that linamarase produced by microorganisms contribute to the process of detoxification of cassava pulp during fermentation (Kimaryo *et al.*, 2000).

2.6 Probiotic microorganisms involved in cassava fermentation for gari

Fermentation process or technology is known and used by many people as one of the oldest forms of food conservation and preservation in different part of the world (Savadago, *et al.*, 2016). The activity of microorganisms during or after fermentation process may protect foods against spoilage and eliminate other antinutritional compounds and it can also increase the shelf-life of many food products (Savadago, *et al.*, 2016). Preservation and protection of foods occurs through lactic acid, alcoholic, acetic acid and high salt fermentations (Nuraida, 2015). Lactic acid bacteria (LAB) and yeast involved in food fermentation or production may possesses probiotic properties and contribute for food quality. Among them *Bifidobacterium* and *Lactobacillus* genera are recognized having high potential in promoting good health (Savadago, *et al.*, 2016). It is well known that Lactic acid bacteria (LAB) are able to retard spoilage, preserve food as well as improve flavour and texture of foods. These microorganisms synthesize a variety of antimicrobial compounds such as organic acids, hydrogen peroxide, diacetyl and bacteriocins which are important in fermented food characteristics and properties (Savadago, *et al.*, 2016).

(Fuller, 2016) defined a probiotic as a live microbial feed supplement which beneficially affects the host animal by improving its intestinal microbial balance.

Probiotics are live microorganisms which, when administered in adequate amounts, confer a health benefit on the host (Hill *et al,* 2014).

FAO/WHO also defined probiotics as a live microorganisms which when administered in adequate amounts confer a health benefit on the host (Reid, 2005).

2.7 **Microbes present in commercially sold gari**

There are reports on the high load of microorganisms in gari sold in the market (Amadi and Adebola, 2008). Which includes: *Salmonella spp.*, *Klebsiellaspp.*, *Pseudomonas spp.*, *Bacillus spp.*, *Clostridium spp.*, *Fusarium spp.*, *Aspergillus spp*; *Penicillium* spp., *Rhizopus spp* and *Cladosporium spp* Olopade *et al.*, (2014).

According to Olopade *et al.* (2014), study, thirty-six gari samples (eighteen each of white and yellow types) were subjected to microbial analysis. Samples were serially diluted to 10^4 and appropriate dilutions inoculated by spread plate method onto Nutrient agar, MacConkey agar and Potato-Dextrose agar plates for Total aerobic plate count (TAPC), Coliform count(CC) and Fungal count respectively.. TAPC for white gari and yellow gari ranged from $1.1x10^4$ to 9.6×10^3 and 1.0×10^3 to 6.0×10^2 respectively, coliform count for white and yellow gari ranged from no growth (NG) to 8.0×10^2 and no growth (NG) to 9.0×10^2 respectively. fungal count of white and yellow gari ranged from no growth to $6.0x10^2$ and 9.0×10^2.

The bacteria isolated from the various samples include *Bacillus spp Enterobacter spp, Pseudomonas, Staphylococcus* and *Klebsiella spp.* Fungi isolated includes *Aspergillus niger, Aspergillus fumigatus, Fusarium, Rhizopus and Penicillium spp*. The pH of the samples ranged from 4.76 to 4.94 in the yellow type and 4.78 to 4.91 in the white type. The moisture content was 6 to 8 percent in yellow type and 4 to 7 percent in the white type which are of low value Olopade *et al.*, (2014).

According to Orji *et al.* (2016), study on Bacteriological Quality and Public Health Implications of Fermented Cassava (Gari) Sold in Okwor and Nkalagu Markets in Ebonyi State, Nigeria. Sixteen (16) samples (8 white and 8 yellow) were purchased from two markets and processed using standard procedures. One gram (1g) of each sample of gari were homogenized in 9ml of sterile distilled water (10^{-1} dilution), further serial dilution of sample homogenate to 10^{-4} was carried out also in a sterile distilled water, transferring 1ml of initial

suspension into subsequent tubes used for the serial dilution. Approximately, a 0.1ml aliquot of appropriate dilution (10^{-4}) was spread on plates of MacConkey agar for coliform count and on Nutrient agar for total Aerobic Plate Count (TAPC) of yellow and white gari samples respectively. All culture plates were incubated at (37°C aerobic) for 24hrs. It was reported that the gari samples studied had high pH which ranged from 5.54 to 6.64 and moisture content of 11.85 to 15.95. Total Aerobic Plate Count (TAPC) for white gari and yellow gari ranged from No growth (NG) to 9.2×10^6 and 1.07×10^1 to 8.3×10^8 respectively. The percentage occurrence of organism isolated in their study include Staphylococcus *aureus* which was the most isolated bacterium (43.8%) from both markets, followed by *E. coli* (18.8%), *Bacillus cereus* (15.6%), *Pseudomonas aeruginosa* (12.5%) and *Streptococcus* spp. (6.2%). *Yersinia* spp. showed the least occurrence with only one isolate with 3.1% frequency (Orji *et al.*, 2016).

Also, in the research of Fawole *et al.* (2016), on Microbiological and Nutritional Evaluation of Gari during Storage at Ambient Temperature. The samples constitute of freshly prepared garri (0 hours), 2 weeks, 4 weeks and 8 weeks old gari. The samples were plated out using Nutrient agar and MacConkey agar by pour plate method, where the identification procedure showed that a total of nine microorganism were isolated from the gari sample. This consisted of four bacterial isolates which were confirmed as *Bacillus sp, Staphylococcus sp, Streptococcus sp and Serratia sp* with reference to Bergeys manual of determinative microbiology. The five fungal isolates were confirmed as *Aspergillus flavus, Aspergillus niger, Aspergillus sp, Penicillium sp* and *Rhizopus sp.* The Moisture content of sample ranged from 7.03 at 0 hours to 8.97 at 8 weeks.

Also, according to Mofolorunsho *et al.* (2016), study on Mycoflora and Moisture Content of Gari Sold in Anyigba, Kogi State. Ten samples of white and yellow gari were collected from sellers in Garage market and Anyigba old market which are the two major open markets in Kogi State. Samples were serially diluted to 10^4, 10^5 and 10^6 and appropriate dilutions inoculated by spread plate method onto Potato Dextrose Agar for viable fungal counts. Plates were incubated for 72 hours at 25°C. Mean total fungal counts (TFL) was 2.03×10^4, 2.20×10^5, and 6.40×10^6 for white gari and 3.96 x 104, 8.80 x 105 and 8.60 x 106 for yellow gari were reported on their study. Moisture contents of white and yellow gari samples ranged from 18.0 – 23.0% and 20.0 – 25.0% respectively.

It was reported that five mould species were isolated from both white and yellow gari samples, which include *Aspergillus* spp., *Rhizopus* spp., *Penicillium* spp., *Mucor* spp. And *Neurospora* spp. it was also reported that yellow gari harbour higher mould species.

According to Ogugbue *et al.* (2011), study on Assessment of microbial air contamination of post processed gari on sale in Port harcourt markets. 50g of gari samples were purchased from four different markets and distributed into three batches. Batch A samples consisted of openly displayed gari in sterile basins kept in selected food shops for a period of one month without any form of handling, Batch B samples were openly displayed in sterile basins but covered with an Econet SF mesh (hole size, 0.30 mm × 0.75 mm) to screen out insects and fomites while Batch C which was used as control were kept in hermetically sealed high density polyethylene bags to exclude the influence of the ambient environment. Ten grams of each sample were aseptically weighed into 90 ml of 0.1% (w/v) sterile peptone water in a sterile 500 ml beaker and allowed to stand for 5 min with occasional stirring using a magnetic stirrer, Thereafter, 10-fold serial dilutions of samples were made and 0.1 ml aliquot of each dilution was plated on nutrient agar for total culturable heterotrophic bacterial (TCHB) counts and potato dextrose agar supplemented with 0.1% concentrated lactic acid for total fungal counts. The plates were incubated at 37°C for 24 h for bacteria and at 25°C for 3 to 5 days for fungi. After incubation, distinct colonies that developed were enumerated and expressed as colony-forming units per gram (CFU/g) of sample. The bacteria and fungal IMA levels were 55 to 85 CFU/dm2 /h) which was said to be above the acceptable limit (50 CFU/dm2 /h). It was also reported that Increase in bacterial and fungal loads of the displayed samples during the period of study ranged from 2.78 to 5.62 LogCFUg-1 and from nil to 4.74 LogCFUg-1 respectively.

The bacteria isolated from the various samples include *Bacillus spp., Salmonella spp., Pseudomonas aeruginosa, Escherichia coli, Micrococcus spp., Acinetobacter spp., Achromobacter spp. and Staphylococcus spp.,* while the fungal isolates obtained include species belonging to the following genera: *Aspergillus niger, Geotrichum candidum, Penicillium notatum, Rhodotorula spp., Fusarium spp., Mucor spp. and Cephalosporium spp.*

2.8 Effect Of Some Microbes Found on Gari in Human Body

Various groups of moulds have reported to be associated with gari during storage and distribution (Ogiehor *et al.,* 2012). In addition, processing steps such as sun-drying and solid-state fermentation coupled with storage of gari could provide favourable conditions for the growth of moulds and the production of mycotoxins (Abu et al., 2010). Aflatoxins B1, B2, G1, G2 are the most frequently encountered mycotoxins because they are produced by ubiquitous fungal genera such as *Aspergillus* and *Penicillium* (Ogiehor *et al.,* 2012).

Exposures to aflatoxins through ingestion of contaminated foods and inhalation of toxins have been linked to acute and chronic toxicity in animals. Effects such as acute liver damage, liver cirrhosis, induction of tumors and teratogenic and other genetic effects in animals and humans have been reported (Ogiehor *et al.,* 2012).

Microorganism, especially bacteria vary from species to species in nutritional requirement (Asegbeloyin and Oyimonyi, 2007). Their presence in food at any stage depend on the nutritional status of the food at any stage, temperature, water content, pH as well as the nature of the organism (Egbuobi *et al.,* 2015).

Microorganism associated with food exposed to environment are *Salmonella typhi* which was incriminated in salmonella food poisoning outbreak in Germany and Great Britain in 1988 and 1971 respectively (Egbuobi *et al.,* 2015). *Shigella flaxner* associated with food contaminated with faecal material. Others include *Bacillus cerus, Pseudomonas spp.; Clostridium spp.; Klebsiella spp.; and Staphlococcus aureus* (Ijebadeniyi, 2007).

CHAPTER THREE

3.0 RESEARCH METHODS AND MATERIALS

Materials: The apparatus that was used for this experiment are; pipette, disposable petri dish, test tube rack, conical flasks, beakers, digital balance, foil paper, masking tape, spatula, cotton wool, spirit lamp, marker and measuring cylinder, Nutrient agar, Macconkey agar, potato dextrose agar, Salmonella shigella agar, distilled water, Gram stain kits, Antimicrobial susceptibility test kit.

3.1 **Study area**

The study was carried out in three markets (Oye, Ilupeju and Ayede) located in Oye local government area in Ekiti state, Nigeria. The markets in these three villages usually observe their market days every 5 days. Oye is a Local Government in Ekiti state bounded by ilupeju Local Government to the North, Irepodun/Ifelodun to the South, Ikole Local Government to the East and Ido/Osi Local Government to the West.

It comprises of the following towns and villages: Oye Ekiti, Ilupeju Ekiti, Ayegbaju Ekiti, Ire Ekiti, Itapa Ekiti, Osin Ekiti, Ayede Ekiti, Itaji Ekiti, Imojo Ekiti, Ilafon Ekiti, Isan Ekiti, Ilemeso Ekiti, Omu Ekiti, Ijelu Ekiti, Oloje Ekiti, and a host of others.

There are no distinct ethnic groups in the Local Government as a greater percentage of the people are of the Yoruba Language race. Nearly all the people speak Yoruba Language race with negligible dialectical variations.

3.2 Sample collection

Gari samples both white and yellow was randomly obtained from different sellers in the three markets (Oye, Ilupeju and Ayede) and also one sample as control from gari processing factory at araromi along Oye road. The control sample was collected immediately it was removed from frying pan using a sterile desicator which was used to cooled it before transferring to sterile polythene bag to avoid melting of polythene bag due to high temperature (hotness). Each sample was collected in a separate polyethene bag with appropriate labelling to indicate color of sample and area of collection, sample number, date and time of collection. Eight (four (4) white, four (4) yellow) samples was collected from each of the markets (Oye, Ilupeju and Ayede) market. A total of twenty five (25) samples was collected and was analysed in the laboratory before 24 hours.

3.3 Determination of pH

The pH of the sample was determined following the method describe by Olopade *et al.*, (2014). In this, 1g of each sample was homogenised in 9ml of distilled water and the pH of the suspension was determined using a reference glass electrode pH meter.

3.4 Moisture Content Determination

The moisture content of each sample was determined by using method described by Mofolorunsho *et al.* (2016), Ten grams of each sample was weighed and dried in an oven at 100°C for 1 hour after which, It was placed in desiccators to cool and then reweighed. This was repeatedly weighed until a constant weight was obtained. The moisture content was then determined by finding the difference in weight.

3.5. Preparation of Media

Nutrient Agar (NA), Macconkey Agar, Potato Dextrose Agar (PDA) and Salmonella Shigella agar was used for the isolation of microbes in the samples. The above media was strictly prepared based on manufacturer instruction and was then autoclave at 121^0C for 15 minutes.

3.6 Preparation of sample

About 1g of each sample was aseptically mixed and weighed using a weighing balance, followed by preparation of 9ml of sterile distilled water in ten test tubes. 1g of the sample was used to make a 10 fold serial dilution by transfering successively 1ml aliquot of the supernatant into 9ml of sterile distilled water.

3.7 Inoculation of sample

About 0.1ml of appropriate dilution was aseptically plated using pour plate technique for total viable bacterial count on Nutrient agar, total coliforms count on Mcconkey agar, Salmonella Shigella agar, total viable fungi count on Potatoe dextrose agar. The inoculated plate was allowed to set and then incubated at 37°C for 24 hours for Nutrient agar, Mcconkey agar plate and Salmonella Shigella agar. Potato dextrose agar was incubated at 25°C for 48 hours. After incubation period, the colonies that developed on the plates was counted and recorded as colony forming unit per gram of sample (CFU/g). Representative of the isolates/colony was subcultured in a fresh plate to get pure culture of the isolates.

3.8 Identification of microbial isolates

The cultural and biochemical characteristics of the pure culture of the isolates obtained was identified using the protocol described by Adetunji *et al.,* 2012. The following cultural, morphological and biochemical features was determined;, cellular arrangement Gram's staining, motility test, spore staining, catalase test, methyl red test, starch hydrolysis, citrate utilization, and oxygen reaction etc for proper identification of isolates.

3.9 Antibiotic Susceptibility Test (AST): Antibiotic susceptibility was determined using procedure described by Adams *et al.* (2011), The method make use of agar diffusion technique on Mueller-Hinton agar (Kirby-Bauer NCCLS modified disc diffusion technique) using ten antibiotic discs corresponding to the drugs most commonly used in the treatment of human and animal infections caused by bacteria; Gentamycin(CN) 10ug, Ampiclox(APX) 30ug, Rocephin(R) 25ug, Ciproflocaxin 10ug, Septrin(SXT) 30ug, Streptomycin(Strep) 30ug , Amoxacillin(AM) 30ug, Augumentin(AU) 30ug, Pefloxacin(PEF) 30ug, and Zinnacef 20ug.

Table 1 shows the pH and Moisture content of white and yellow types of gari samples obtained from three major markets (Oye, Ayede and Ilupeju) in Ekiti state Nigeria. It review that most of the samples had pH and Moisture content within the limits of 5.90-6.82 and 1.00-1.58. The highest pH was observed in sample Q (yellow gari) collected from Ilupeju market with moisture content of 1.33 while the lowest pH was observed in sample W (white gari) collected from Ilupeju market.

Table 2 shows the total heterotrophic count (THPC) of white and yellow types of gari samples obtained from three major markets in (Oye, Ayede and Ilupeju), Ekiti state Nigeria. The table review that the total heterotrophic count of white gari samples ranged from 2 $\times 10^4$–5 $\times 10^4$CFU/g and for yellow gari samples ranged from 2 $\times 10^4$– 7$\times 10^4$ CFU/g. The fungi count of white gari ranged from 2 $\times 10^2$ –8 $\times 10^2$CFU/g while fungi count of yellow gari ranged from 1$\times 10^2$– 7$\times 10^2$ CFU/g, There was no presence of coliforms on the gari samples collected from the three (3) markets (Oye, Ayede and Ilupeju). The highest THPC was observed from yellow (Q) gari sample collected from Ilupeju market with microbial load of 7 $\times 10^4$ CFU/g, while the lowest was 2 $\times 10^4$ observed from gari samples collected from Oye market (E, F, H), Ayede market (J, K, L, M, N, P) and from Ilupeju market (R, S, T). The highest fungi count was observed in white gari sample collected from ilupeju market (V) with fungi count of 8 $\times 10^2$CFU/g while the lowest fungi count was observed from yellow gari sample collected from Oye market (B) with fungi count of 1$\times 10^2$ CFU/g. Table 2 also reveals that the gari samples were contaminated by diverse microbial spp mainly of *Bacillus subtilis, Bacillus cereus, Bacillus macerans, Serratia marcescens, Bacillus licheniformis, Asperillus fumigatus, Asperillus niger, Asperillus flavus, Bacillus polymyxa, Micrococcus roseus, Pseudomonas. aeruginosa, Penicillum sp, Asperillus fumigatus.*

Table 3 shows the biochemical reactions and cellular morphology of isolates from three (3) different Market (Oye, Ayede and Ilupeju).

Table 4 Shows the zone of inhibition of different identified bacterial isolates using antibiotics discs. The zone of inhibition of different isolates ranges from 10–35mm. The highest zone of inhibition was observed in plate of *Bacillus subtilis* with Augumentin(AU) 30ug which shows zone of inhibition of 35mm, while the lowest zone of inhibition was observed in plate of *Bacillus Cereus, Staph. aureus, Salmonella typhi* with Amoxacillin(AM) 30ug, Septrin(SXT) 30ug, (Ampiclox(APX) 30ug and Amoxacillin(AM) 30ug and Zinnacef 20ug), then Ciproflocaxin 10ug with zone of inhibition 10mm respectively.

CHAPTER FOUR

RESULTS

4.0 TABLE 1: PHYSICOCHEMICAL PROPERTIES OF GARI SAMPLES

SAMPLE	PH	MOISTURE CONTENT
YELLOW		
OY A	6.75	1.13
OY B	6.73	1.42
OY C	6.75	1.58
OY D	6.72	1.23
WHITE		
OY E	6.78	1.21
OY F	6.77	1.25
OY G	6.80	1.12
OY H	6.77	1.13
YELLOW		
AY I	6.64	1.34
AY J	6.67	1.08
AY K	6.68	1.49
AY L	6.68	1.46
WHITE		
AY M	6.64	1.37
AY N	6.68	1.30
AY O	6.62	1.42

AY P	6.68	1.00
YELLOW **IL Q**	6.82	1.33
IL R	6.09	1.33
IL S	6.35	1.28
IL T	6.80	1.25
WHITE **IL U**	6.70	1.36
IL V	6.69	1.24
IL W	5.90	1.30
IL X	5.95	1.42

KEY; IL= Ilupeju, AY= Ayede, OY= Oye

4.1 TABLE 2: TOTAL HETEROTROPHIC COUNT OF ISOLATES FOUND ON GARI SAMPLES

SAMPLE	THPC	COLIFORM	FUNGI COUNT	ORGANISMS ISOLATED
YELLOW				
OY A	6×10^4	**NIL**	2×10^2	*B. subtilis, B. cereus, B. macerans, Serratia marcescens A. niger, A. Flavus*
OY B	3×10^4	**NIL**	1×10^2	*B. subtilis, B.licheniformis, Serratia marcescens A. niger, A. flavus, A. Fumigatus*
OY C	6×10^4	**NIL**	3×10^2	*B. subtilis, B. cereus, B. macerans, B. licheniformis, A. fumigatus, A. niger, A. Flavus*
OY D	6×10^4	**NIL**	3×10^2	*B. polymyxa, B.cereus, B. licheniformis, A. niger, A. flavus*
WHITE				
OY E	2×10^4	**NIL**	2×10^2	*B. licheniformis, P. aeruginosa, Serratia marcescens, B. subtilis, A. niger, A. flavus, Penicillum sp.*
OY F	2×10^4	**NIL**	4×10^2	*B. subtilis, P. aeruginosa, B. licheniformis, A. niger, A. flavus, A. fumigates*
OY G	4×10^4	**NIL**	2×10^2	*B. subtilis, P. aeruginosa, B. licheniformis, A. niger, A. Flavus*
OY H	2×10^4	**NIL**	2×10^2	*B. subtilis, P. aeruginosa, B. licheniformis, A. niger, A. flavus, A. Fumigates*
YELLOW				*B. polymyxa, B.cereus, Serratia*

AY I	3×10^4	**NIL**	3×10^2	*marcescens, B. subtilis, A. niger A. flavus*
AY J	2×10^4	**NIL**	6×10^2	*Micrococcus roseus, B. subtilis, P. aeruginosa, B.cereus, A. niger*
AY K	2×10^4	**NIL**	3×10^2	*B. polymyxa, B. subtilis, B.cereus, A. flavus, A. Niger*
AY L	2×10^4	**NIL**	4×10^2	*B. polymyxa, B.cereus, B. subtilis, A. niger, A. flavus, Penicillum sp.*
WHITE AY M	2×10^4	**NIL**	6×10^2	*B. polymyxa, B.cereus, B. subtilis, P. Aeruginosa, A. niger, A. flavus, Penicillum sp.,*
AY N	2×10^4	**NIL**	5×10^2	*B. macerans, B.cereus, B. subtilis, A. niger, A. flavus, Penicillum sp.,*
AY O	4×10^4	**NIL**	6×10^2	*B. polymyxa, B.cereus, B. subtilis, , P. aeruginosa, A. flavus, A. Niger*
AY P	2×10^4	**NIL**	6×10^2	*Serratia marcescens, B. licheniformis, B.cereus, B. subtilis, A. niger, A. flavus, Penicillum sp*
YELLOW IL Q	7×10^4	**NIL**	3×10^2	*Serratia marcescens, P. aeruginosa, B.cereus, B. subtilis, Penicillum sp, A. fumigatus, A. Niger*
IL R	2×10^4	**NIL**	7×10^2	*Micrococcus roseus, B. polymyxa, B. licheniformis, B.cereus, P. aeruginosa, A. niger, Penicillum sp*
IL S	2×10^4	**NIL**	4×10^2	*Micrococcus roseus, B.*

				polymyxa, B. licheniformis, P. aeruginosa, A. fumigatus, A. niger, Penicillum sp
IL T	2×10^4	**NIL**	7×10^2	*Serratia marcescens, B. polymyxa, B. licheniformis, P. aeruginosa, A. fumigatus, , A. niger, A. Flavus*
WHITE **IL U**	5×10^4	**NIL**	7×10^2	*Serratia marcescens, P. aeruginosa, B.cereus, B. subtilis, penicillum sp, A. fumigatus, A. Niger*
IL V	4×10^4	**NIL**	8×10^2	*Salmonella sp., B. polymyxa, P. aeruginosa, Staph. aureus, Penicillum sp, A. niger, A. fumigatus, A. Flavus*
IL W	3×10^4	**NIL**	5×10^2	*Serratia marcescens, B. polymyxa, P. aeruginosa, Staph. aureus, Penicillum sp, A. niger, A. fumigatus, A. Flavus*
IL X	4×10^4	**NIL**	5×10^2	*B. polymyxa, P. aeruginosa, B. cereus, Staph. aureus, B. subtilis, Penicillum sp, A. flavus, A. Fumigatus*
C0NTROL	**NIL**	**NIL**	NIL	NIL

KEY: THPC= Total Heterotrophic Count, NILL=No Growth, IL = Ilupeju, AY= Ayede, OY= Oye

4.2 TABLE 3: IDENTIFICATION OF ISOLATES USING BIOCEMICAL TEST AND CELLULAR MORPHOLOGY

ISOLATE CODE	FRU	REF	MAL	MAN	GAL	LAC	SUC	GLU	SPOR ST	URE	CITR	NITR	GEL	STAR	MRT	MOT	INDO	OXI	CAT	GRAM	CELLULAR MORPHOLOGY	PROBABLE ORGANISM
1	+	–	–	–	–	–	–	+	+	ND	+	+	+	+	–	–	–	+	+	+ve	Rods	*Bacillus cereus*
2	+	+	+	+	+	+	+	+	+	ND	+	+	+	+	–	+	–	+	+	+ve	Rods	*Bacillus polymyxa*
3	+	–	+	+	–	–	–	+	–	ND	–	+	–	–	–	+	–	+	+	+ve	Cocci	*Micrococus luteus*
4	+	–	–	+	+	+	+	+	+	ND	+	+	+	+	–	+	–	+	+	+ve	Rods	*Bacillus subtilis*
5	+	+	+	+	–	–	+	+	–	ND	–	+	+	–	–	+	–	+	+	_ve	Rods	*P. aeruginosa*
6	+	–	–	+	+	–	+	+	+	ND	+	+	+	+	–	–	–	+	+	+ve	Rods	*Bacillus licheniformis*
7	+	–	+	+	+	–	+	+	–	ND	+	+	+	ND	–	+	–	–	+	_ve	Cocci	*Serratia marcescens*
8	+	–	+	+	+	–		–	ND	ND		–		–		–	–	ND	+	+ve	Cocci in clusters	*Micrococcus luteus*
9	+	+	+	+	+	+	+	+	+	ND	+	+	+	+		+		+	+	+ve	Rods	*Bacillus macerans*
10	ND	–	+	ND	ND	–	–	+	–	ND	–	+	–		+		–	–	+	_ve	Rods	*Salmonella typhi*
11	+	–	–	–	–	–	+	+	+	ND	+	–	–	+	+	–	–	–	–	+ve	Rods	*Clostridium perfringens*
12	+	–	+	–	+	+	+	+	–	ND	+	+	+	ND	+	–	–	–	+	+ve	Cocci in clusters	*Staph. aureus*

KEY: FRU=Fructose, REF=Reffinose, MAL=Maltose, MAN=Mannose, GAL=Galactose, LAC= Lactose, SUC= Sucrose GLU= Glucose, SPOR ST= Spore Staining, URE= Urea test, CITR= Citrate Utilisation, NITR= Nitrate test, GEL= Gelatin Hydrolysis, STAR= Starch Test, MRT= Methyl Red Test, MOT= Motility Test, INDO= Indole Test, OXI= Oxidase Test, CAT= Catalase test, GRAM= Gram reaction.

Sensitivity Test Using Antibiotics

Method: Disc (agar) diffusion method

4.3 Table 4: Inhibition zone diameter

Antibiotics	***Bacillus subtilis***	***Bacillus Cereus***	***Staph. aureus***	***Salmonella Typhi***	***Bacillus polymyxa***	***Clostridium Perfringens***	***Sereitia Marcescens***	***Pseudomonas aerugi nosa***	***Micrococcus luteus***	***Bacillus Marcerans***
Gentamycin(CN) 10ug	15	19	17	20	12	14	18	16	19	20
Ampiclox(APX) 30ug	18	14	10	15	18	12	15	11	17	18
Rocephin(R) 25ug	20	16	15	17	22	21	26	15	22	26
Ciprofloxacin 10ug	20	11	13	10	14	16	22	13	20	23
Septrin(SXT) 30ug	12	10	11	11	16	11	16	11	16	14
Streptomycin(Strep) 30ug	23	18	11	21	15	16	23	17	13	17
Amoxacillin(AM) 30ug	10	14	10	18	13	16	21	15	19	17
Augumenti										

n(AU) 3Oug	35	25	23	28	20	19	28	29	24	27
Pefloxacin(PEF) 30ug	15	17	14	22	21	17	29	23	22	25
Zinnacef 20ug	14	13	10	20	12	13	17	12	14	12

CHAPTER FIVE

DISCUSSION

A total of seven bacterial genera isolates and two fungal genera were obtained from both white and yellow gari samples in this study. The microbial isolates include: *Bacillus polymyxa, Bacillus licheniformis, Pseudomonas aeruginosa, Bacillus cereus, Bacillus subtilis Staphylococcus aureus and Bacillus marcerans, Aspergillus niger, Aspergillus fumiatus, Aspergillus flavus and penicillium spp.* Several reports from various researchers have shown the isolation of some of these microorganisms in gari samples and other fermented foods (Ogiehor *et al.* 2007; Olopade *et al.* 2014; Mofolorunsho *et al.* 2016; Orji *et al.* 2016).

The alarming rate of death and ill health caused by food poisoning, individuals, families, health care system and society as well as commercial enterprises incur tremendous economic loss. These losses include the cost of medical care, the cost of investigating food contamination outbreaks, legal costs and fine (Orji *et al.* 2016). The microbial content of food and food products depicts interplay between pH and moisture contents. The results of this present study showed that the pH of white and yellow gari samples analyzed ranged from 5.90 – 6.80 and 6.09 – 6.82 respectively while the moisture content of white and yellow gari ranged from 1.00 – 1.42 and 1.08 – 1.58 respectively. This study validates the work of Olapade *et al.,* 2014 who also reported that low pH and moisture content reduce the growth of bacteria in the gari samples.

According to ICMSF 2014, microbial counts of $\leq 10^3$ are satisfactory, while counts of 10^4 to $< 10^5$ is acceptable. The sample with the highest microbial counts of 7×10^4 with pH of 6.82 is from Ilupeju market and this is still within the acceptable count. This result is in line with the work of Olapade *et al* 2014 who obtained an acceptable range of plate counts, but contrary to the result of the study carried out and reported by Orji *et al.,* 2016. The microbial

counts obtained from this study can be attributed to the inability of microorganisms to thrive in low pH, low moisture and prevailing conditions in the gari.

The presence of coliforms generally signifies poor sanitary condition in the production of gari. There was no presence of coliform in this study which negates the results of Olapade *et al.,* 2014 who observed high presence of coliform in their study. This could be due to the fact that there are no dumping sites or public toilets close to the markets in this study unlike the studies carried out by Olapade *et al.*, 2014 and Orji *et al.,* 2016.

The presence of *Bacillus cereus* and *Staph aureus* calls for concern because some strains of these organisms are known to be toxigenic and have been implicated in food borne intoxication (Olapade *et al.,* 2014). *Bacillus cereus* is common environmental contaminants while *Staph aureus* is of human origin, their presence could therefore be from the food handlers, utensils and the environment (Olapade *et al.,* 2014).

The high rate of occurrence and distribution of moulds such as *Aspergillus flavus, Aspergillus niger,Aspergillus fumigatus* and others may be traced to the inadequate post processing handling practices, the ubiquitious nature of these fungi, and their ability to withstand and tolerate harsh environmental conditions such as low pH and low moisture content of gari (Ogiehor *et al.,* 2007). These species of *Aspergillus* are known to produce deleterious mycotoxins under favourable conditions (Oranusi *et al.,* 2013; Olopade *et al.,* 2014; Ogiehor *et al.,* 2007), their presence in gari must therefore be treated with caution.

According to the study conducted by Tamba *et al.,* 2016, the antibiotic resistance pattern of 14 species of *Salmonella* isolated from raw and fermented milk showed 81.8% resistance pattern of the antimicrobial agents used. The authors also reported that the Minimum Inhibitory Concentration (MIC) test showed that only 1(7.1%) of the isolate was sensitive to Amoxicillin with < 0.12 while the remaining 9 (98.9%) were resistant to the two antibiotics used. Narges *et al.,* (2018) also reported a high level a resistance of *Salmonella* sp to a wide

range of commonly used antibiotics due to inappropriate use of drugs, hence salmonellosis is a global public health problem. In this study, Salmonella is also resistance to some of the antibiotics used with Septrin(30ug) being the least reactive of them. Augumentin(30ug) is the most effective of the antibiotics and *Salmonella*sp is susceptible to it with a zone of diameter of 28mm.

CONCLUSION AND RECOMMENDATION

In conclusion, microbial contamination of gari is as a result of practices associated with post processing of this product. These processes includes spreading the finished products on the mat to cool, storage condition, displaying in open bowl in the markets during sales and some customers frequent touch before buying which may contribute considerably to the microbial burden of gari sold in markets in Ekiti state. Some of the organism isolated in this study can cause food borne disease and production of mycotoxins in the case of fungi that can have serious health effect in human; it is henceforth important to develop a strategy to properly package and store this product to reduce microbial contamination.

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