



Microbiological Identification and Characterization of Pathogenic Bacteria in Garri Sold within the University of Benin

*¹Aghemwenhio, I.S., ¹Chukwu, F.O., ³Ogbebor, V., & ²Akeem, S.A.

¹Department of Microbiology, University of Benin, Benin City, Edo State, Nigeria.

²Department of Agricultural Technology, Edo State College of Agriculture and Natural Resources

³Department of General Studies, Edo State College of Agriculture and Natural Resources

*Corresponding author email: sandraitohan18@gmail.com

Abstract

Garri is one of the processed cassava products. It is a staple cuisine for most people in southern Nigeria and is consumed widely throughout West and Central Africa. Nigeria alone produces an estimated ten million tons annually. Processing conditions and storage containers have the ability to act as growth enhancers for bacteria and fungi, resulting in microbial contamination of garri. Pathogenic bacteria and opportunistic pathogens can create toxins and other metabolites that can result in infectious diseases, which are one of the main causes of death globally each year. For this reason, it is crucial to have extremely sensitive, specific, and quick approaches for identifying and managing foodborne infections as possible health hazards. In the University of Benin, the student market (June 12 market) is the place where different foodstuffs like rice, beans, yams, garri etc are made available for students' consumption and the hygiene practices of the market sellers is of great concern. Data on the attitude and knowledge regarding hygiene and sanitation level of the food handlers actively selling in the market was unknown, hence the need to conduct a research on the hygiene, sanitation and microbiological quality of the food handlers in the market, (June 12 market) University of Benin. The following bacteria, *B. cereus*, *Salmonella*, *Shigella*, *E. coli* were isolated from garri samples obtained from the student market at the University of Benin. The findings of the observation indicated that there were a lot of pathogenic bacteria in the food sample (garri), which may have been caused by the market workers' poor hygiene and sanitization standards, as well as those of the distributors and processors. These bacterial pathogens can be prevalent in a particular geographical location though they could arise anywhere based on hygiene standard of food handlers at all levels.

Keywords: Pathogenic Bacteria, Garri, Food Safety, University of Benin, Bacteria

Introduction

In tropical Africa, Nigeria inclusive, grows cassava and it is among the most important staple food crops grown in the region. Globally, Nigeria is among the current leading cassava producing countries and cassava plays a major role in efforts to alleviate the African food crisis because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions, and suitability to present farming and food systems in Africa (Chinwe et al., 2016). In the past, several techniques have been used to process cassava roots into a variety of products, which are then used in different ways based on regional tastes and customs. Nigeria alone produces an estimated ten million tons of garri annually (Okafor et al., 2018). One popular processed product made from cassava (*Manihot esculenta* Crantz) tubers is garri. In Nigeria and other West African nations, garri is among the cassava products that are often consumed (Awoyale et al., 2021). One of the most popular cassava products is garri, which is inexpensive, quick to prepare, and not easily perishable (FAO, 2010; Oluwafemi and Udeh, 2016). According to Adebayo et al. (2012). Okolo and Makanjuola (2021), the majority of cassava harvested from Nigerian farms is processed into garri. The methods for obtaining garri vary depending on the region and intended use; however, the general process for the commercial production of garri entails harvesting the matured roots of the cassava plant, peeling the roots, washing the peeled roots, grinding the washed roots, de-watering the mash, fermenting the mash, wet sieving the fermented mash, dry frying/roasting, open air-cooling on a floor or mat, and packaging for distribution (Okafor et al., 2018; Okolo & Makanjuola, 2021).

Processing conditions and storage containers have the ability to act as growth enhancers for bacteria and fungi, resulting in microbial contamination of garri (Adebayo et al., 2012; Akindele & Abimbola, 2018). Microorganisms, including fungus and bacteria, are important for public health (Okolo & Mekanjuola, 2021). From stored, retailed, and ready-to-eat garri from several communities in Nigeria, microorganisms such as *Fusarium* sp., *Aspergillus* sp., *Salmonella* sp., *Shigella* sp., and *Escherichia coli* have been isolated (Ogbonna et al., 2017). Today's world places a premium on food safety because it affects public health worldwide. Any food's microbiological risks should be taken into consideration while evaluating its danger. The microbiological criteria in the system of food safety characteristics show if food products are fit for ingestion. In addition, the type or mode of processing and the degree of production cleanliness are indicated by the qualitative and quantitative microbial composition found in raw materials and ready-to-eat products (Elshafei 2017). For this reason, it is crucial to have fast, highly sensitive, and specialized approaches for identifying and managing foodborne infections as possible health risks (Liu et al., 2019). As of right now, most homes and communities still consume garri mainly untreated, which puts some consumers at risk for health problems related to microbes and their toxins (Okafor et al., 2018). The purpose of this study is to ascertain the microbiological quality of the given food sample (Garri) sold within University of Benin (June 12 market).

Materials and Methods

This research was carried out on the main campus of the University of Benin, Benin City, Edo state, Nigeria. The campus accommodates students in its ten university halls; Halls 1-6 for undergraduate students, Halls 7-8 and Akingbola Hostel for post-graduate students and Intercontinental hostel for international students studying at the University of Benin. There are also off-campus private hostels in areas around the campus such as Ekosodin, BDPA and Osasogie. The project work was undertaken at Central Research Laboratory, University of Benin. The food sample (garri) was collected from the designated location (June 12 market) in University of Benin. A sample was collected from 3 shops at June 12. Collected samples were kept in sterile containers in the laboratory in preparation for analysis. Materials such as Petri dishes, pipettes, glass containers (conical flask, round bottom flask), and bottles were drained and dried. They were wrapped with aluminum foil and sterilized in a hot-air oven at 160°C for an hour. They were allowed to cool after sterilization before usage. An aseptic working environment was achieved using a Bunsen burner flame and the disinfection of work surfaces with alcohol. All media used were obtained from Oxoid and were prepared according to manufacturers' instructions. The media used in this study include; Nutrient agar, MacConkey agar and *Salmonella-Shigella* agar (SSA). 28g of Nutrient agar was weighed then dissolved in 1000ml distilled water. It was autoclaved afterwards at 121°C for 15 minutes. The agar was allowed to cool down to 45-50°C before aseptically introduced into the petri dishes. 23.2g of MacConkey agar was weighed and dissolved in 500ml distilled water then autoclaved at 121°C for 15 minutes. The agar was allowed to cool down to about 45-50°C before aseptically introduced into the petri dishes. 12.0g of SSA was weighed and dissolved in 256ml distilled water then autoclaved at 121°C for 15 minutes. The agar was allowed to cool to around 45-50°C before aseptically introduced into sterile petri dishes. This agar was used to further distinguish the colourless/pale colonies seen on MacConkey agar. 1g of each garri sample (labelled A,B,C) was put into sterile containers, distilled water was added to emulsify and the serial dilution process took place. The dilution was a 10 fold dilution in five test tubes for each sample. The plating technique used was Pour plate method and this was carried out in a sterile environment to avoid contamination which could disrupt the expected results. The poured media were allowed to solidify then incubated for 24hours at 37°C. Afterwards, the number of bacteria on each plate was counted using a colony counter and manual counting. The organisms were subcultured using MacConkey agar by streaking the isolate on the solidified agar surface and incubated at 37°C for 24hrs. The colourless colonies seen on MacConkey agar were further distinguished by sub-culturing using *Salmonella-Shigella* agar. This was achieved by streaking the selected colony onto a sterile SSA plate and incubating at 37°C for 24 hours. The colonies appeared colourless after incubation but some formed black centers within the colonies as a result of production of Hydrogen sulphide (H₂S). Pure cultures of the bacterial isolates were obtained from the subculture of a single colony from the successful pour plate technique and were characterized using cultural, morphological and biochemical methods. Several tests, such as Gram staining, catalase, urease, Triple Sugar Iron test, oxidase and citrate tests were carried out to identify bacterial isolates presumptively. A Gram staining test was carried out to determine the presence of Gram-positive and Gram-negative isolates. Neat, grease-free and sterile-dried microscope slides with labels were smeared using a sterilized loop, and the organism was air-dried and heat-fixed over a flaming Bunsen burner. The fixed smear was saturated with drops of crystal violet, left for one minute to react and washed off with distilled water. Lugol's iodine, which serves as a mordant, was added, left for one minute, and washed off with distilled water. The smear was decolourized with 95% ethyl alcohol, went for 30 seconds and washed off with distilled water. Then, the streak underwent counter staining using safranin solution for one minute and was rinsed with distilled water. Lastly, the smear was allowed to air

dry and immersion oil was added for a microscopic view on an immersion objective lens light microscope. Colours, shapes and arrangements were used in identifying the organisms. Gram-positive organisms maintained the crystal's purple colour, while Gram-negative retained the pink of safranin. To better characterize these isolates, biochemical tests were conducted, which included: The urease test is used to identify bacteria capable of producing the urease enzyme. The organisms that secrete urease can hydrolyze urea to ammonia and carbon dioxide. This test was used to distinguish urease-positive bacteria from other Enterobacteriaceae. The isolated pure bacteria were inoculated into well-prepared and autoclaved Christensen-modified urea broth and incubated for 24 hours at 37°C. Urease-positive cultures produced a pink colour due to a change in the indicator's colour in the presence of ammonia, while the negative result remains no colour change or yellow-orange colour. The citrate utilization test is a part of the test used to differentiate organisms on their ability to utilize citrate as the primary energy source. A citrate test was performed to differentiate members of Enterobacteriaceae capable of fermenting citrate in the presence of the enzyme citrate. Simon's citrate agar contained citrate as significant energy and was prepared for inoculation on Petri dishes. Well-prepared and sterilized citrate agar plates were inoculated from the pure isolated culture by streaking the surface with a sterilized loop. The plates were then incubated at 37°C for 24 hours. There were changes in colour due to bacterial growth of the organisms on the medium due to citrate metabolism, which gave a positive citrate test. The shift in pH turns the bromothymol blue indicator in the medium from green to blue (positive result). A negative test was demonstrated with no growth, no colour change, or the colour of the medium remains green. The oxidase test was carried out to detect the presence of a cytochrome oxidase or indophenol oxidase that will catalyze electrons between electron donors in the bacteria and a redox dye known as tetramethyl-p-phenylene-diamine. The dye would be reduced to deep purple colour if yielded to positive reactions. Several reagents can be used for this study, but Kovacs oxidase reagent: 1% tetra-methyl-p-phenylenediamine dihydrochloride in water, was used. The filter paper was saturated with a Kovacs oxidase reagent solution, and a speck of the pure culture was smeared on it with a platinum loop. It was allowed and observed for colour development within 10 – 60 seconds. The appearance of a deep purple-blue/blue colour indicated oxidase production, and the negative result was when no colour changed. The Triple Sugar Iron (TSI) test is an ability to test an organism's capability to ferment sugars and to produce hydrogen sulphide (H₂S) or gas (O₂), or both. The test was used primarily to differentiate members of the Enterobacteriaceae family based on their sugar fermentation patterns and from other Gram-negative rods. An agar slant prepared of a TSI agar was used in carrying out this test in a sterile test tube at a slanted angle. The slanted medium was inoculated with TSA pure culture using a straight inoculation needle by stabbing first through the center to the bottom of the tube and streaking the agar slant's surface. After inoculations, the test tubes were covered with foil paper and left at an ambient temperature of 36°C to incubate for 24 hours. Reactions on test tubes were examined, and sugar fermentations were indicated by the production of H₂S, gas and a change in colours from red (alkaline) to yellow (acid). When an alkaline/acid (red top/yellow bottom) slant reaction appeared, it only indicated dextrose (glucose) fermentation. When an acid/acid (yellow top/yellow bottom) slant reaction appeared, it showed the fermentation of dextrose, lactose and/or sucrose. The appearance of an alkaline/alkaline (red top/red bottom) slant reaction represented the absence of sugar fermentation. The blackening of the medium in the slant indicated H₂S production. Bubbles, cracks, or bottom-raised space in the slanted agar indicated gas production (formation of CO₂ and H₂). This test was used to distinguish between bacteria that produce the catalase enzyme, such as *Escherichia coli*, and bacteria that do not, such as *Salmonella*. Catalase catalyzes the breakdown of hydrogen peroxide (H₂O₂) to oxygen (O₂) and water (H₂O). In this test, 2ml of hydrogen peroxide solution was poured into a test tube, and some colonies of the test organism were picked and immersed into the H₂O₂ solution using a sterile glass rod. The bacteria that generated catalase (positive result) produced gas bubbles (oxygen), but those that did not possess catalase enzyme had none (negative result).

Results

Table 1 shows the bacterial load present in little quantity of the food samples from different locations. For ready- to- eat foods, the colony counts are considered high.

Table 1: Colony count of bacteria isolated from garri samples

Dilution	Number of colonies (cfu/g) sample A	Number of colonies (cfu/g) sample B	Number of colonies (cfu/g) sample C
----------	-------------------------------------	-------------------------------------	-------------------------------------

10^{-1}	uncountable	uncountable	uncountable
10^{-2}	no distinct colonies	uncountable	uncountable
10^{-3}	61	76	82
10^{-4}	51	48	55
10^{-5}	38	40	45

Key

Cfu/g: colony forming units per gram

The number of visible colonies of bacteria from garri samples is shown in Table 1

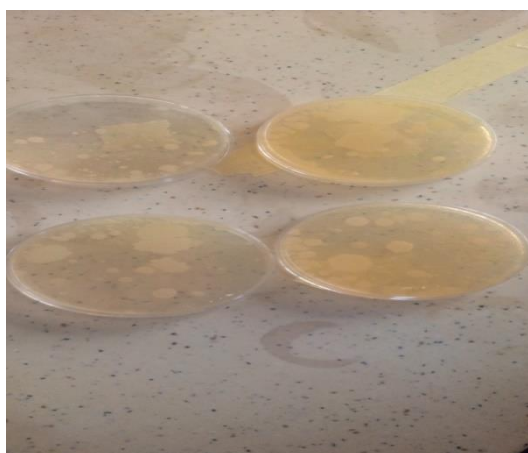


Figure 1: Bacterial growth after incubation

The growth of bacteria after incubation is shown in figure 1



Figure 2: Number of colonies using a colony counter

The use of a colony counter to count the number of colonies is shown in Figure 2

Cultural characteristics of isolates from samples are shown in Tables 2, 3 and 4. This was carried out to identify and differentiate microorganisms based on their growth patterns, colony morphology, and other observable traits.

Table 2: Cultural characteristics of isolates from samples A,B,C on Nutrient Agar

SIZE	FORM	ELEVATION	MARGIN	COLOUR
Large	Circular	Flat	Entire	Greyish-white
Large	Circular	Slightly raised	Irregular	Cream
Small	Circular	Flat	Regular	Opaque
Large	Circular	Slightly raised	Entire	Cream

The cultural characteristics of isolates from the three samples are shown in Table 3

Table 3: Cultural characteristics of isolates from samples A, B, C on MacConkey Agar

SIZE	FORM	ELEVATION	MARGIN	COLOUR	MICROORGANISM SUSPECTED
Small/ medium	Round	Slightly raised	Entire	Pink/red	<i>Escherichia coli</i>
Medium/large	Round	Flat/ slightly raised	Irregular	Pale/colourless	<i>Salmonella/ Shigella</i>
-----	-----	-----	-----	-----	<i>Bacillus</i>

Table 4 : Cultural characteristics of isolates on *Salmonella – Shigella* Agar

SIZE	FORM	COLOR	LACTOSE FERMENTING	H ₂ S PRODUCTION	MICROORGANISM CONFIRMED
Small	Round and slightly irregular	Colourless/ pale	+ve	+ve	<i>Salmonella</i>
Small/ medium	Round	Pale/colour less	-ve	-ve	<i>Shigella</i>

Table 5 shows the result of gram staining which was performed to access bacteria presence and classify them based on their cell wall properties.

Table 5: Result of gram staining conducted on isolates

S/N	COLOUR OBSERVED	SHAPE	SUSPECTED ORGANISM
1.	Red /pink	Cylindrical straight slightly curved rod	<i>Escherichia coli</i>
2.	Red/pink	Cylindrical straight slightly curved rod	<i>Salmonella</i>
3.	Red/pink	straight slightly curved rod	<i>Shigella</i>
4.	Purple	Rod shaped	<i>Bacillus</i>

Biochemical tests were performed to differentiate specific bacteria species and to aid in understanding microbial ecology as shown in Table 6

Table 6: Biochemical tests conducted on isolates

S/N	UREASE TEST	CITRATE TEST	OXIDASE TEST	TSI TEST	CATALASE TEST	MICROORGANISM
1.	-ve	-ve	-ve	Acid/acid	+ve	<i>Escherichia coli</i>
2.	-ve	-ve	-ve	Acid/acid	+ve	<i>Salmonella</i>
3.	-ve	-ve	-ve	Alkaline/acid	-ve	<i>Shigella</i>
4.	+ve	+ve	-ve	Alkaline/alkaline	+ve	<i>Bacillus</i>

Discussion

In the current global context, food safety is a critical issue for public health. Any food's microbiological risks should be taken into consideration while evaluating its danger. Pathogenic bacteria have possibilities of contamination in any step of food processing. Thus, while choosing ingredients, storing and processing food, as well as serving and preserving cooked food, food quality and the sterile process, two crucial principles of food handling, must be put into practice (Li et al., 2020). Sanitation and hygiene are attempts to prevent illness. They are required to keep bacteria that cause disease and contaminate food. In order to offer safe and healthful food, food handlers and those in charge of establishments (particularly public locations) that sell food and drinks should pay close attention to these matters. The findings of the observation indicates that there were a lot of pathogenic bacteria in the food sample (garri), which may have been caused by the market workers' poor hygiene and sanitization standards, as well as those of the distributors and processors.

Table1 shows the bacterial load present in just a tiny quantity of the food sample from different locations, that number of bacteria is more than enough to cause foodborne illnesses when consumed. Cultural characteristics of isolates revealed that the suspected and confirmed Microorganisms in the food sample (garri) were *Salmonella*, *Shigella*, *Bacillus* and *Escherichia coli* which is similar to the report of Obi et al., (2022) who reported that *Bacillus* spp and *Shigella* spp. were present in garri sample from Bida. Obi et al., (2022) also reported that *Streptococcus* spp. and *Micrococcus* spp. were also present in garri. This shows that gram positive and gram negative bacteria can be seen in garri from any location.

Table 2 and 3 further shows the characterization of the mixed colonies seen, this is to identify the microorganisms, trace the illness it causes and determine treatment. Other tests were carried out for further analysis such as Gram staining and biochemical tests. The four microorganisms discussed (*B. cereus*, *Salmonella*, *Shigella*, *E. coli*) all cause diarrhea differently and manifest differently in different people (infants, adults, immunocompromised, etc). *Escherichia coli* belongs to the Enterobacteriaceae family and is a Gram-negative, bar-shaped structure with flagella. It is typically propagated by fecal contamination, motile by peritrichous flagella, and frequently detected within the Gastrointestinal Tract (GIT) of humans and vertebrate animals. It can also be found as a result of contamination during food animal slaughter, but most often, it is found in soil, water, and food due to fecal contamination. *E. coli* is the most common cause of cholecystitis, bacteremia, cholangitis, UTI, traveler's diarrhea, septicemia, newborn meningitis, and other extra intestinal illnesses. Improper handwashing after toilet use, contaminated water supply, poor sanitation during animal slaughter are linked hygiene failures and food safety implications includes risk of severe gastrointestinal and systemic infections, cross contamination in food processing and preparation and unsafe drinking water and irrigated produce. *Salmonella* is also gram negative, it causes food borne illnesses and can survive in dry environments like garri. Garri contaminated with salmonella can lead to gastro intestinal infections. *Salmonella* is associated with contaminated water, food and poor hygiene, crowded living conditions and shared utensils also increase the risk of cross contamination. *Shigella* is Gram- negative and non lactose fermenting. It causes dysentery and it is associated with poor hygiene and faecal- oral transmission. It's presence in garri indicates contamination and potential health risks. Close quarters, communal spaces, and inadequate sanitation contribute to *Shigella* presence, lack of hand hygiene, poor sanitation in communal areas, inadequate washing of raw materials are linked hygiene failures and the food safety applications are direct links to dysentery, fecal-oral transmission and public health risks in densely populated communities. *Bacillus* species are Gram-positive spore forming bacteria, *Bacillus* species are widespread in soil and dust. Some *Bacillus* species are harmless soil inhabitants, while others can cause food spoilage and it's of safety concern because the specific

bacillus species matters; some produce toxins. The presence of these microorganisms in garri samples warrant attention. Proper food handling, cooking, storage of garri in clean dry containers and hygiene practices are essential to ensure safety. Hygiene education, Food handling procedures such as Good Manufacturing Practices (GMPs) and Hazard Analysis and Critical Control Points (HACCP) should be followed to guarantee food safety because contamination can occur at any stage of the process, from harvesting to consumption. Key hygiene practices to mitigate risks include hand hygiene, water quality control, proper storage and surface sanitation.

Conclusion

Foodborne illnesses are a worldwide concern. To identify and control any new foodborne issues that endanger public health and international trade, all nations and the pertinent international organizations must work together. It is crucial to have extremely sensitive, specific, and quick approaches for identifying and managing foodborne infections as possible health hazards. The food business, public health organizations, government regulators, and consumers must work together constantly to stop food contamination in farms, processing facilities, dining establishments, and residential settings. The majority of foodborne infections may be avoided even if their biology, epidemiology, and analysis are complicated. Undoubtedly, a diverse range of disciplinary knowledge and abilities is needed. The food business, public health organizations, government regulators, and consumers must work together constantly to stop food contamination in farms, processing facilities, dining establishments, and residential settings.

Recommendations

1. Appropriate food safety education initiatives for food vendors, food handlers and farmers is highly recommended to reduce the number of foodborne disease incidents. Implementing traceability systems like adopting digital tracking methods to monitor food from source to shelf ensures swift response to contamination events. Enforcing hygiene protocols by regular staff training and facility audits can minimize the risk of microbial contamination. Investing in rapid testing technologies by leveraging portable diagnostics help detects pathogens before distribution.
2. For regulators, harmonizing inspection protocols and safety criteria across regions to reduce trade barriers and protect public health through standardize food safety guidelines.

References

- Adebayo, B.A., Nanam, T.D., Bamidele, E.A., & Braima, D.J. (2012). Quality management manual for the Production of garri. *International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria*, 8, 1–41.
- Akindele, S.T., & Abimbola, W.A. (2018). Microbial evaluation of garri sold in Ijebu community. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT)*, 12(7), 35–38.
- Awoyale, W., Oyedele, H., Adenitan, A.A., Alamu, E.O., & Maziya-Dixon, B. (2021). Comparing the functional and pasting properties of garri and the sensory attributes of the eba produced using back slopped and spontaneous fermentation methods. *Cogent Food and Agriculture*, 7(1), 188–197.
- Chinwe, O.U., Ozumba, I.C., Adejumo, O.A., Ayuba, O.L., Nwosu, C., Bosa, S.O., & Idowu, O. (2016). Sensory and comparative analysis of ordinary garri and cocos garri (Nutritionally enriched). *International Journal of Research Studies in Agricultural Sciences (IJRSAS)*, 2(8), 27–32.
- Elshafei, A.M. (2017). Role of microorganisms in food contamination, processing and safety. *Journal of Food Microbiology*, 1(1), 1–2.
- Food and Agriculture Organization (FAO) (2010). Agriculture Statistics for Food and Agriculture Organization. *News Bulletin*.
- Li, Y., Yang, X., Zhang, H., Jia, H., Liu, X., Yu, B., & Yang, D. (2020). Prevalence and antimicrobial susceptibility of Salmonella in the commercial eggs in China. *International Journal of Food Microbiology*, 325, 108623.
- Liu, Y., Cao, Y., Wang, T., Dong, Q., Li, J., & Niu, C. (2019). Detection of 12 common food-borne bacterial pathogens by TaqMan real-time PCR using a single set of reaction conditions. *Frontiers in Microbiology*, 10, 222.
- Obi, P.U., Mohammed, Y.M., Okeke, K.S., Ibrahim, N.J., Ajayi, M.A., Benedict, A.U., & Umar, M. (2022). Microbial analysis and sensory attributes of garri produced and marketed in Bida, Niger State, Nigeria. *Journal of applied Science and Environmental Management*, 26 (6), 1007–1013
- Ogbonna, I.O., Agbowu, B.I., & Agbo, F. (2017). Proximate composition, microbiological safety and heavy metal contaminations of garri sold in Benue, North-Central Nigeria. *African Journal of Biotechnology*, 16 (18), 1085–1091.

- Okafor Arthur, C., Aquaowo Uwakmfon, A., Ojiagu Kingsley, D., & Agu Kingsley, C. (2018). Preliminary studies on processed Garri as a source of bacterial hazards to students. *Immunology and Infectious Diseases*, 5(3), 25-29.
- Okolo, E., & Mekanjuola, A.T. (2021). Microbial evaluation of garri sold within Ahmadu Bello University main campus, Samaru, Zaria, Kaduna State. *Science World Journal*, 16(3), 259-265
- Oluwafemi, G.I. , & Udeh, C.C. (2016). Effect of fermentation periods on the physicochemical and sensory properties of garri. *IOSR Journal Environmental Science Toxicology and Food Technology*, 10 (1), 37- 42.