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**Scholars Bulletin**
Abbreviated Key Title: Sch Bull
ISSN 2412-9771 (Print) |ISSN 2412-897X (Online)
Scholars Middle East Publishers, Dubai, United Arab Emirates
Journal homepage: https://saudijournals.com

**Subject Category: Biochemistry**

# Chemical and Biological Analysis of Processed Cassava *Garri* Sold in Some Selected Local Government Areas in Kebbi State: A Food Safety Perspective

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**DOI:** https://doi.org/10.36348/sb.2024.v10i10.002 | **Received:** 10.11.2024 | **Accepted:** 06.12.2024 | **Published:** 17.12.2024

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## Abstract

Cassava *garri* is the most common food consumed in West Africa sub-region with more than 70% of the product harvested in Nigeria. This study was aimed to analyze the chemical and biological properties of processed *garri* marketing in Kebbi State for food safety perspective. Nutritional and anti-nutritional factors of the *garri* samples were analyzed using standards methods. Heavy metals were analyzed using atomic absorption spectroscopy. Bacteria and fungi were also isolated and identified. DNA was extracted from *E. coli* and *A. flavus* and was successfully used for PCR. The results revealed that the proximate shows moisture (12.6±0.94 %), crude fiber (5.56±0.08 %), Ash (2.96±0.12 %) and crude protein (2.09±0.16 %) had the highest percentage whereas crude fat (0.77±0.02 %) had the least percentage. The anti-nutritional factors shows that cyanides had the highest content of (111.8±2.28 mg/100g) compare to alkaloid (10.7±3.66 mg/100g) and phytates (2.11±0.34 mg/100g), whereas the oxalates had the least contents of (0.18±0.03 mg/100g). The heavy metals shows that cadmium (Cd) 0.73±0.09 ppm, lead (Pb) 0.61±0.35 ppm and asenic (As) 0.25±0.02 ppm had the highest concentration, whereas mercury (Hg) had the least concentration 0.19±0.02 ppm. A 338 cultured (100%) of bacteria were identified: the highest frequency was recorded from *Escherichia coli* 136(40.2 %), *Micrococcus luteus* 129(38.2 %) and *Streptococcus pneumonia* 39(11.5%), while the least was recorded from *Staphylococcus aureus* 34(10.1 %). A 26 cultured (100%) of fungus were identified: with *Aspergillus flavus* 10(38.5 %), *Aspergillus niger* 8(30.8 %), *Aspergillus fumigatus* 3(11.5 %) and *Aspergillus clavatus* 3(11.5 %), had the highest frequency of isolation, whereas *Rhizopus sp* 2(7.7 %), had the least frequency of isolation. The amplified products obtained with primers specific for both *Escherichia coli* and *Aspergillus flavus* were 900 bp and 700 bp. The study suggests that people should avoid regular intake of *garri* without a proper thermal treatment so as to minimize disease/intoxication before consumption.

**Keywords:** *Garri, Proximate Properties, Antinutritional Factors, Heavy Metals, Bacterial and Funga Isolation, Molecular Analysis*.



## INTRODUCTION

*Garri* is the most common cassava-based food consumed in West Africa sub-region. More than 70% of cassava harvested in Nigeria is processed into *garri* (Adebayo *et al.,* 2012). It is recognized as a roasted granular hygroscopic starchy food product from cassava (*Manihot esculenta crantz*). Rapid increase in the population throughout the sub-saharan Africa is currently being accompanied by demand of convenient foods (Qstergard *et al.,* 2015). Cassava therefore, provides ideal raw materials for many food products due to their easy processing techniques. The cassava root contains cyanide which is poisonous but varies from one variety to the other (Qstergard *et al.,* 2019). Roots that contain high level of cyanide taste bitter which are not eaten raw while the roots of sweet varieties contain low level of cyanide and can be eaten (James *et al.,* 2019). *Garri* is the most preferred cassava product because it is less expensive, less bulky, easy to cook and not readily perishable (FAO, 2010; Samuel and Ugwuanyi 2014; Oluwafemi and Udeh, 2016). White and yellow *garri* are two common varieties of *garri* in the Nigeria markets. Hydrogen cyanide produce from enzymatic degradation of cyanoglysides, a potential toxicity resulting in acute cyanide poisoning include drop in blood pressure, dizziness, headache, vomiting, diarrhea, blue discoloration due to the lack of oxygen (cyanosis), twitching and convulsions. Anti-nutritional factors are chemical compounds synthesized in natural foods by the normal metabolism of species. Some of these chemicals are known as ‘‘secondary metabolites’’ and they have been shown to be highly biologically active. Ogugbue *et al.*, (2017) observed that the current method of selling *garri* in most local markets may pose potential risks to public health.

Consumers are unaware of the chemical and biological hazards associated with some of the trace chemical constituents present in food samples (Eaton and Croopman, 2008; Hayes, 2016). *Garri* has been classified as a “street food” which refers to a wide variety of ready-to-eat foods sold, and sometimes prepared in because it is stored and marketed in a ready public places. It is a convenient product because it is stored and marketed in a ready public places (Mensah *et al.,* 2012). Chitosan is linear polysaccharides generally prepared by alkali or enzymatic N-deacetylation of chitin (Dutta *et al.*, 2012). Chitosan have been applied in the food industries due to its functional characteristics such as bioconversion, preservatives and antimicrobial activity (Chung *et al.*, 2021). The antimicrobial action of chitosan is influenced by numerous intrinsic factors, such as its sources (insect shells, fungi, concentration, molecular weight that generates its type and polymerization degree (Batista *et al.,* 2023). Cyanogenic glucosides, linamarin and lotaustralin are potentially toxic compounds, that the cassava plant contains (Tewe and Iyayi, 2006; Mburu *et al.*, 2012), of which the amount of these toxic compounds varies according to cultivars and climatic conditions (Mburu *et al.*, 2012). Improper preparation of bitter cassava cause a disease called *KONZO* (Ayodeji, 2014). Phytate and oxalates easily forms insoluble complexes with copper, iron and calcium. Tannins chelate metals, reduce the absorption of these nutrients, and also inhibit the action of digestive enzymes (Cock, 2016). In Nigeria, the consumption pattern varies according to ecological zones. *Garri* is a roasted granule, is the dominant product and is widely accepted in both rural and urban areas. It can be consumed without any additives or it can be consumed with a variety of additives such as sugar, groundnut, fish, meat and stew. The north east also uses the cassava as fufu/akpu, *garri* abacha.

Heavy metals are referred to as metals having atomic weight greater than sodium, and possess some level of toxicity (AdepojuBello *et al.*, 2014). Heavy metals could be toxic and essential: Toxic heavy metals mainly Pb, Cd, Hg and As can cause metal poisoning to the patients. Some heavy metals are essential and required by the human body in trace amounts. However they become toxic when blood level increased. They may cause damage to vital organs of the body like heart, liver, kidneys and brain (Uddin *et al.,* 2016). *Garri* as a cassava tuber should be free from microbial contamination *garri* should be processed within two days of harvest to prevent deterioration or loss of quality in *garri*. Air-borne contaminants in the market areas were found to contribute to the micr5obial burden of *garri* sold in the market. *Garri* is creamy-white granular flour with a slightly fermented flavor and a slightly sour taste made from fermented gelatinized fresh cassava tubers. Fresh cassava tuber is most, low acid food that is susceptible to bacteria and fungi growth. Hygienic practices especially in the early stage of processing should therefore ensure minimal contamination, all waste material from the process should be removed from the site as they are produced to avoid contamination of the final product with peel sand. The study provides view into nutritional value of processed *garri* for human consumption. The study also will contribute to find out whether proper hygiene practices are carried out during processing of *garri* to avoid the risk of contamination. Therefore, this study is aim to conduct the chemical and biological analysis of processed *garri* sold in some selected local government areas in Kebbi State: a food safety perspective, and is targeted in determine the nutritional, anti-nutritional, heavy metals, bacteria and fungi and finally molecular analysis of the isolate in *garri* sold in the market.

To date *garri* is still being consumed largely in Kebbi State without considering the harmful effects it might cause ranging from poor processing and the storage methods. However, due to high demand of *garri* by consumers the producers of *garri* what they after this day is the money they will make from the sale of the product. Thus the problem of the study therefore is to find out whether proper hygiene practices are carried out following during the processing of *garri* to avoid the risk of contamination. Mode of storage, transportation, handling and conditions could serve as the critical points of contamination of *garri*, example bacteria if not properly processed and fungi if not properly stored. Moreover, there is a fear that high intake of foods that are rich in anti-nutrients may expose the body to potentially harmful compounds. According to the World Health Organization, there are some crucial mistakes that individuals should steer clear of when preparing and drinking *garri* such mistakes include; poor hygiene, unsafe water, unbalanced diet and inadequate soaking time.

Rapid increase in the population throughout the sub-saharan Africa is currently being accompanied by demand of convenient foods (Qstergard *et al.*, 2015). Cassava therefore, provides ideal raw materials for many food products due to their easy processing techniques. However, the fleshy part is bulky and costly to transport from one place to the other, which are likely to rot within few days if left unprocessed. The cassava root contains cyanide which are poisonous but varies from one variety to the other (Qstergard *et al.,* 2019). Roots that contain high level of cyanide taste bitter which are not eaten raw while the roots of sweet varieties contain low level of cyanide and can be eaten (James *et al.,* 2019).

The health benefits of *garri* is not neglected when it comes to its consumption, but the health implications of *garri* has raised eyebrows to the increased consumption of this great food. Regular intake of *garri* might cause ulcer due to the high levels of cyanide in the poor processed *garri*. Cyanide which is an organic acid in nature when it's more concentrated in the intestine leads to increased risk of developing ulcer. Furthermore, hydrocyanic acid in *garri* can cause serious eye defect. It can increase blood sugar level in diabetic patients. This may cause these patients various health risks like infertility and reduced wound healing ability. This study targeted at determining the prevalence of toxic chemicals, bacteria and fungi in *garri* being sold in the market. The study was touches the key aspects which including: nutritional factors, anti-nutritional factors, heavy metals contamination, microorganisms, molecular analysis and safety for human consumption in Nigeria.

*Garri* are widely consumed cassava product among people of Kebbi State, Nigeria and many part of the African countries. Qualities of *garri* produced in Nigeria such as high beta-carotene content which is a precursor to Vitamin A, excellent *garri* and fufu quality, regular shapes etc., are unique selling propositions that should come in handy in the processing and storage methods. It is therefore of great important to not only identify the culprits involved in *garri* but also to ensure that processed *garri* is safe for human consumption. Therefore, there is need to process *garri* to reduce the cyanide level, moisture content to a safe level and also increase the shelf life for more than two years without any significant changes in quality (flavor, colour, odour and texture) which may be cause by molds. People should also avoid foods that are high in added sugars, salt and unhealthy fats. Soaking and boiling help in releasing the toxic hydrogen cyanide before consumption. G*arri* mixed with palm oil can help to improve poor eyesight because of the vitamin A in palm oil (WHO, 2009).

Furthermore, in order to reduce the occurrence of *garri* contamination, *garri* sellers need to be educated on better methods of *garri* handling and storage. Processor/retailers should be made aware of the danger of improper storage of *garri*. Moreover, monitoring committee's in collaboration with some standard organizations in Nigeria should be promoted for safety and human consumption. Detection and measurement of compositions in *garri* is very important, in order to compare levels of contamination with the recommended residue limit, so that appropriate remedial action can be taken and appropriate preventive agents of *garri* contamination during handling and storage are implemented. The research covered some selected local government areas in Kebbi State: Argungu, Bimin Kebbi and Jega. Nevertheless the research sample is limited to the processed *garri* only sold in the selected areas. The aim of this study is to conduct the chemical and biological analysis of processed *garri* sold in some selected local government areas in Kebbi State: A food safety perspective. The objectives of the study were a. o determine the presence of nutritional and anti-nutritional factors in *garri* sample, to detect the presence of some heavy metals in *garri* sample, to determine the cyanide content using rapid test in *garri* sample, to isolate and identify bacteria and fungi associated with *garri* samples, and to extract DNA and amplified with the help of PCR using selected isolates of bacteria and fungi.

## MATERIALS AND METHODS

### *General Procedure and Laboratory Analysis*

Commercially *garri* were collected from three (3) different selected local government areas in Kebbi State namely Argungu, Birnin Kebbi and Jega. Twenty gram (20g) of each sample was for laboratory analysis. Proximate analysis and anti-nutritional factors were determined based on the procedure described in Association of Official Analytical Chemists procedures (AOAC, 2023). Heavy metals were determined using atomic absorption spectroscopy (AAS) (Omokehide *et al*., 2013). Rapid test for cyanide detection in *garri* was carried out (Haque and Bradbury, 2015). The Potato Dextrose Agar (PDA: 200g) was used for the isolation of fungi from *garri* sample. Fungal isolates were identified using Czapek Dox Agar (CDA) for macroscopic identification, while the microscopic features were identified using slide culture techniques (Fujita, 2019). Bacterial identification was based on morphological characteristics (colony appearance, shape, elevation, edges, consistency, colony surface and pigmentation) (Cheesbrough 2018). DNA Extraction, PCR reactions for the detection of the Bacteria and fungi were based on the general procedure described in Agersborg *et al.,* (2012).

### Data Analysis

The data to be generated from the study was presented as mean± SD and was subjected to one-way analysis of variances (ANOVA), and the statistical difference between the mean was evaluated using Duncan's Range Test at $p<0.05$.

## RESULTS

### Nutritional and Anti-Nutritional Factors in *Garri* Sample

#### *Proximate Composition of Garri Sample*

The proximate (%) shows that moisture (12.6±0.94 %), crude fiber (5.56±0.08 %), Ash (2.96±0.12 %) and crude protein (2.09±0.16 %) had the highest percentage whereas crude fat (0.77±0.02 %) had the least percentage. Nitrogen free extractives which is the difference between the sum of the amounts of ash, crude protein, crude fat and crude fiber was also calculated (Table 1), and the result is supported by the values obtained from previous studies of Ogbonna *et al*., (2017).

**Table 1: Proximate composition (%) of *garri* sample**

| S/N | Sample Labeled | %Moisture | %Ash | %Crude Fiber | %Crude Protein | %Crude Lipid | Nitrogen Free Ext. |
|---|---|---|---|---|---|---|---|
| 1 | B/Kebbi | 11.3±2.86 | 2.36±0.46 | 5.56±0.08 | 2.07±0.05 | 0.77±0.02 | 89.2±4.01 |
| 2 | Jega | 12.6±0.94 | 2.06±0.94 | 3.86±0.12 | 1.71±0.03 | 0.73±0.03 | 91.64±0.00 |
| 3 | Argungu | 10.6±1.88 | 2.96±0.12 | 4.43±0.16 | 2.09±0.16 | 0.74±0.04 | 89.7±3.68 |

There were no statistically significant difference since the P-value is greater than 0.05 means (P>0.05).

***Anti-Nutritional Factor of Garri Sample***

The anti-nutritional factors (mg/100g) results shows that cyanides (111.8±2.28 mg/100g) was present in huge quantity, compare to alkaloid (10.72±3.66 mg/100g) and phytates (2.11±0.34 mg/100g), whereas the oxalates had the least contents of (0.18±0.03 mg/100g) (Table 2), and the result obtained is consistent with previous report of Sarkiyayi *et al*., (2010).

**Table 2: Anti-nutritional factors (mg/100g) of *garri* sample**

| S/N | Sample Labeled | Oxalate | Phytate | Cyanide | Alkaloid |
|---|---|---|---|---|---|
| 1 | B/Kebbi | 0.18±0.03 | 1.41±0.19 | 107.0±3.98 | 9.50±1.31 |
| 2 | Jega | 0.14±0.09 | 2.11±0.34 | 102.3±6.20 | 10.3±0.39 |
| 3 | Argungu | 0.12±0.02 | 1.83±0.19 | 111.8±2.28 | 10.7±3.66 |

At (P>0.05) it judged as no significant difference observed because P-value is greater than 0.05 mean

***Heavy Metals of Garri Sample***

The heavy metals results shows that cadmium (Cd) 0.73±0.09 ppm, lead (Pb) 0.61±0.35 ppm and asenic (As) 0.25±0.02 ppm had the highest concentration, whereas mercury (Hg) had the least concentration 0.19±0.02 ppm (Table 3), and the result obtained is similar with previous literature report of Omokehide *et al*., (2013).

**Table 3: Heavy metals of processed *garri* sample**

| S/N | Sample Labeled | Pb | Cd | Hg | As |
|---|---|---|---|---|---|
| 1 | B/Kebbi | 0.23±0.14 | 0.73±0.09 | 0.17±0.04 | 0.23±0.01 |
| 2 | Jega | 0.61±0.35 | 0.68±0.15 | 0.19±0.02 | 0.25±0.02 |
| 3 | Argungu | 0.45±0.31 | 0.68±0.37 | 0.15±0.02 | 0.21±0.02 |

No significant difference observed since the P-values were greater than 0.05 means (P>0.05).

***Cyanide Content Using Rapid Test in Garri Sample***

Cyanide content results were observed based on the color change (Table 4), with Argungu had the highest positive followed by Jega and Birnin Kebbi, and these result is similar with previous report of Haque and Bradbury, (2010).

**Table 4: Screening of *garri* for cyanide detection**

| Sample Labeled | Result |
|---|---|
| B/K1 | + |
| B/K2 | ND |
| B/K3 | ND |
| JG1 | + |
| JG2 | ND |
| JG3 | ND |
| ARG1 | + |
| ARG2 | + |
| ARG3 | ND |

Key: += Positive ND= Not Detected

**Identification of Bacteria and Fungi Associated with *Garri* Samples**

***Frequency and Percentage Occurrences of Fungal Isolate***

A 26 cultured (100%) were identified: with *Aspergillus flavus* 10(38.5 %), *Aspergillus niger* 8(30.8 %), *Aspergillus fumigatus* 3(11.5 %) and *Aspergillus clavatus* 3(11.5 %), had the highest frequency of isolation, whereas *Rhizopus sp* 2(7.7 %), had the least frequency of isolation (Table 5), and the result is consistent with reference to Brown (2005).

**Table 5: Frequency of isolation and percentage of fungal isolates in *garri* sample**

| S/N | Sample Labeled | Fungus | Frequency | Percentage |
|---|---|---|---|---|
| 1 | Argungu | *Aspergillus flavus* | 10 | 38.5 |
| 2 | Jega | *Aspergillus niger* | 8 | 30.8 |
| | | *Aspergillus fumigates* | 3 | 11.5 |
| 3 | B/Kebbi | *Aspergillus clavatus* | 3 | 11.5 |
| | | *Rhizopus sp.* | 2 | 7.7 |
| **Total** | | | **26** | **100%** |

***Frequency and Percentage Occurrences of Bacterial Isolate***

A 338 cultured (100%) were identified: the highest frequency of isolation was recorded from *Escherichia coli* 136(40.2 %), *Micrococcus luteus* 129(38.2 %) and *Streptococcus pneumonia* 39(11.5%), while the least was recorded from *Staphylococcus aureus* 34(10.1 %) (Table 6), and the result are similar with reference to Okafor *et al.* (2018).

**Table 6: Frequency of isolation and percentage of bacterial isolate in *garri* sample**

| S/No | Sample Labeled | Bacteria | Number of Colonies | Frequency |
|---|---|---|---|---|
| 1 | Jega | *Escherichia coli* | 136 | 40.2 |
| 2 | Argungu | *Micrococcus luteus* | 129 | 38.2 |
| | | *Streptococcus pneumonia* | 39 | 11.5 |
| 3 | B/K | *Staphylococcus aureus* | 34 | 10.1 |
| **Total** | | | **338** | **100%** |

***Biochemical Characterization of Bacterial Isolate***

Biochemical results revealed the presence of *Escherichia coli, Micrococcus luteus, Streptococcus pneumonia* and *Staphylococcus aureus* (Table 7), and the result obtained is consistent with previous literature report of Ugochukwu *et al.*, (2015).

**Table 7: Identification of bacterial isolates from *garri***

| Test Organism | Gram Stain | Shape | Cell | Mot | Gas | Suc | Lact | Glu | Cat | Cit | Ind |
|---|---|---|---|---|---|---|---|---|---|---|---|
| *Escherichia coli* | Gram Negative | Rod | Singly | - | + | + | + | - | + | - | + |
| *Micrococcus luteus* | Gram Positive | Cocci | Cluster | - | - | + | + | + | + | - | - |
| *Streptococcus pneumonia* | Gram Positive | Cocci | Chain | - | + | + | + | + | - | + | - |
| *Staphylococcus aureus* | Gram Positive | Cocci | Cluster | - | - | - | + | + | + | + | - |

Key: += Positive, Suc= Sucrose, Cat= Catalase, Ind= Indole, -= Negative, Lact= Lactose, Cit= Citrate, Mot= Motality, Glu= Glucose

***Cultural Characteristics of the Fungal Isolates***

The following morphological and colonial appearance of fungal species were isolated from *garri* samples showed variation in the colony color, reverse, phialides, conidiophores, conidia, conidial head, vesicle and inference.

**Macroscopic Feature**

| Surface | Reverse | Phialides | Conidiophore | Conidia | Conidial head | Vesicle | Inference |
|---|---|---|---|---|---|---|---|
| -Green | -Black | -Directly on vesicle | Has stipe<br>-Hyaline<br>-Smooth<br>-Yellow green<br>-Aseptate | -Globose<br>- Green<br>-Smooth wall | -Radiate<br>- Green | -Subglobose | *Aspergillus flavus* |
| -Black | -Dark brown | -Directly on metulate | -Has stipe<br>-Hyaline | -Globose<br>-Dark brown | -Radiate | -Subglobose | *Aspergillus niger* |

 

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | | -Smooth<br>-Dark brown<br>-Aseptate | -Smooth wall | | | |
| -Sued | -Dark green | -Directly on green pigment | -Smooth<br>-Dark green<br>-Aseptate | -Globose<br>-Green<br>-Smooth wall | -Columnar | -Broadly clavate | *Aspergill sfumigatus* |
| -Sandy | -Pale brown | -Directly on clavate | -Erect<br>-Simple<br>-Smooth<br>-Pale brown | -Globose<br>-Pale brown<br>-Rough wall | -<br>Cylindrical | -Subglobose | *Aspergillus clavatus* |
| -Grey | -White yellowish | -Directly above the rhizoids | -Branching<br>-Nonseptate<br>-Black | -Globose<br>-White yellowish<br>-Rough wall | -Umbrella-like | -Subglobose | *Rhizopus species* |

***Microbial Counts***

The total microbial counts showed that bacterial counts 8.6 × $10^{-7}$ CFU/g, were higher than the fungal count 5.5 × $10^{-4}$ CFU/g and the results was supported by International Commission on Microbiological Specification for Foods (ICMSF, 2000) as recommended.

**Table 8: Number of colonies in CFU obtained from processed *garri***

| Name of Sample | Dilution Factor | Media Used | Number of Colonies in CFU/ml |
|---|---|---|---|
| *Garri* (*Escherichia coli*) | $10^{-6}$ | NA | 8.6×$10^{-7}$ |
| *Garri* (*Aspergillus flavus*) | $10^{-3}$ | PDA | 5.2×$10^{-3}$ |

Key: NA= Nutrient Agar, PDA= Potato Dextrose Agar, CFU= Colony Forming Unit

***DNA Extraction and Amplified with Help of PCR***

PCR amplicons were produced successfully in all DNA extracted. The amplified products obtained with primers specific for both *Escherichia coli* and *Aspergillus flavus* were 900 bp and 700 bp, respectively, which were the expected product sizes of the amplified gene with the set of primers used. Figure 1 shows a photograph of agarose gel electrophoresis of these PCR amplicons, and the result obtained are similar with reference to Tell *et al.*, (2012).

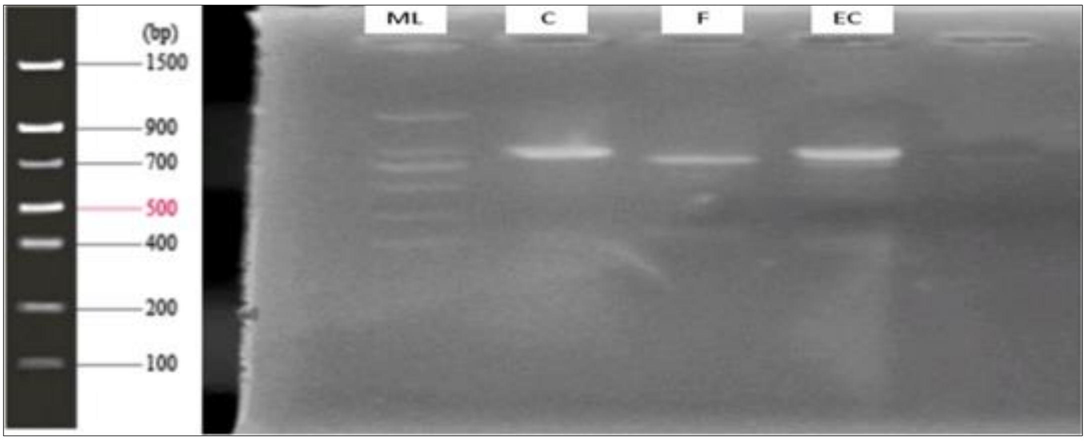


**Figure 1: Agarose gel electrophoresis showing the 900-bp and 700-bp PCR amplicons for *E. coli* and *A flavus* respectively. Lane 4 shows PCR amplicons for *E coli* (E), while lane 3 shows PCR products for *A. flavus* (F) from DNA extracted. Lane 2 serve as control (C), while lane 1 is molecular ladder (ML) from DNA extracted.**

## DISCUSSION

This study examined three samples of cassava *garri* collected from three selected local government in Kebbi State and found that the proximate composition of the *garri* samples varied with moisture 12.6±0.94 %, crude fiber 5.56±0.08 %, Ash 2.96±0.12 % and crude protein 2.09±0.16 % had the highest percentage whereas crude fat 0.77±0.02 % had the least percentage in the *garri*. Foods with high moisture content are highly prone to spoilage because most microorganisms proliferate under such condition. The relatively low moisture level of *garri* in this study will make them less prone to microbial spoilage. IITA (2005), the chemical composition of *garri* shows that it is made up of 62% moisture, 35% carbohydrates, 1% protein, 1% fat and 1% mineral content. Carbohydrates forms 35%, starch forms 84% of it and others metals forms simple sugars. The sugar constituents are made up of sucrose (71%), glucose (13%), fructose (9%) and maltose (3%). Moisture content is an integral part of proximate composition analysis of food (Gemede *et al.*, 2015). It is an index of its water activity and is used as a measure of stability and susceptibility to microbial contamination (Uyoh *et al.*, 2013). Moisture plays a very important role in the keeping quality of foods and high moisture can have an adverse effect on their storage stability. Hence, the reduced moisture content below 13 in the *garri* samples could be a good indication of their prolonged shelf life (Olaoye *et al.*, 2006; Olaoye and Onilude 2008). This implies that *garri* can be stored easily and the moisture content can contribute in slowing down growth and development of microorganisms and in hindering hydrolysis of components present in cassava (Ngaha *et al.*, 2016).

FSANZ (2004) *garri* is rich in starch. It also has very high fiber content. Also contains proteins and some essential vitamins. The high fiber content makes it very filling, and also makes this good in preventing or at least reduces likelihood of constipation and bowel diseases. The low fibre content of the *garri* could also prevent intestinal irritation; improve digestibility and overall increase in nutrient utilization (Odedeji *et al.*, 2014). Okon *et al.*, (2010) reported that a diet low in fibre is undesirable and could cause constipation; hence many diets are associated with disease of colon like piles, appendicitis and cancer. The result, in any case, shows that *garri* can be a good source of protein to meet up with the low quality protein affecting human and animal population in the developing countries (Soetan and Aiyelaagbe, 2016). In addition, *garri* could play a significant in the provision of cheap and affordable protein for rural populations (Ndlovu and Afolayan, 2008). The fat present was probably hydrolyzed to free fatty acid by lipase enzymes produced by the mould leading to decrease in fat content. The relatively low fat in *garri* shows that the cassava can be recommended as weight reducing diet and can therefore be consumed in large quantities with safety without risk of cardiovascular disease, obesity and other related diseases (Okon *et al*, 2017; Loucou *et al.*, 2018).

In addition, according to the report on *garri* specifications, it was documented that *garri* should be enriched with vitamins, proteins and other essential nutrients (Anon., 2009). The relatively low content of protein in this study suggests a need for protein enrichment of *garri* before consumption. This indicates possibility of its usage as a supplement of other protein sources in food (Siddig, 2009). Nitrogen and protein content of the two samples are in agreement with that reported for other polysaccharide (Siddig, 2010). Though it shows that, samples could be used to provide protein supplement in both human and animal diet. However, with respect to the moisture and ash contents, findings from this study were within the specified limits (≤ 7 % and ≤ 1.50 %) recommended for *garri* except for one of the locations (Birnin Kebbi) which was slightly above the recommended limit for moisture content. The ash content of the *garri* samples in this study were relatively higher than 1.54 - 1.70 % and 1.5 - 2.4 % reported by Udoro *et al.*, (2014) and Ogbonna *et al.*, (2017) respectively for ash content of *garri* in their studies. The level of ash in food commonly indicates the quality of its mineral contents. The more the ash contents, the higher the mineral contents of the food sample. The level of crude fibre, crude fat and lipid content is in this study are similar to the values obtained from previous studies (Udoro *et al.*, 2014; Ismail *et al.*, 2015; Ogbonna *et al.*, 2017).

Anti-nutritional factors of *garri* sample shows that cyanides was present in huge quantity from 111.8±2.28 mg/100g compared to alkaloid 10.72±3.66 mg/100g and phytates 2.11±0.34 mg/100g, whereas the oxalates had the least contents of 0.18±0.03 mg/100g. Moreover, there is a fear that high intake of *garri* that are rich in anti-nutrients may expose the body to potentially harmful compounds. According to the World Health Organization there are some crucial mistakes that individuals should steer clear of when preparing and drinking *garri* such mistakes include; poor hygiene, unsafe water, unbalanced diet and inadequate soaking time. Cyanide which is an organic acid in nature when it's more concentrated in the intestine leads to increased risk of developing ulcer. There are number of varieties of cassava plants that are generally categorized as 'bitter' or 'sweet' cassava depending on their cyanide content (Navia and Villada, 2012). Thus, the roots of 'bitter' cassava contain relatively high concentration of cyanide; the peels and pulps, on the average, contain about 650 ppm and 310 ppm total cyanide respectively, whereas the corresponding 'sweet' cassava peels and pulps contain less than 200 ppm and 38 ppm total cyanide respectively. As reported by WHO (2009) when *garri* mixed with palm oil can help to improve poor eyesight because of the vitamin A in palm oil. Boiling of *garri* in water with added palm oil before consumption resulted in a decrease in cyanogens level of 96->99% (Ngudi *et al.*, 2010). Consumption of high tropane alkaloids will cause rapid heartbeat, paralysis and in fatal case, lead to death. Uptake of high dose of tryptamine alkaloids will lead to staggering gate and death. Indeed, the physiological effects of alkaloids have on humans are very evident. Other toxic action includes disruption of the cell membrane in the gastrointestinal tract (Fernando *et al.*, 2012). Moreover, using rapid test, cyanide was detected based on the color change using NaOH, $FeSO_4$ and concentrated HCl acid, with Argungu had the highest positive, followed by Jega and Birnin Kebbi.

The presence of oxalate in *garri* above acceptable levels causes irritation in the mouth and the lining of the gut (Abeza *et al.,* 2007) and also hinders the absorption of divalent minerals, particularly calcium (Ola and Oboh, 2000). This in effect makes calcium inaccessible by the body, especially for maintenance of strong bones, teeth, co-factor in enzymic reactions, nerve impulse transmission and blood clothing (Unuofin *et al.,* 2017). If food with excessive amounts of oxalic acid is consumed regularly, nutritional deficiencies are likely to occur, as well as severe irritation to the lining of the gut. A distinctive property of oxalic acid that makes it so dangerous is that once it has linked with calcium, it is practically insoluble at the acidic pH normally found within the body. An unusual characteristic of calcium oxalate is that nothing can dissolve it and that makes it such an exasperating problem. Therefore, people who have tendency to form kidney stones are advised to avoid oxalate-rich foods. On the other hand, people suffering from coronary heart disease are encouraged to consume moderately oxalate rich foods as it helps to reduce blood cholesterol ((Abeza *et al.,* 2007).

Phytate is the salt form of phytic acid known as inositol hexakisphosphate (IP6). It is the principal storage form of phosphorus in many plant tissues, especially bran and seeds. Phytate is not digestible by humans or non-ruminant animals, so it is not a source of either inositol or phosphate if eaten directly. Phytate works in a broad pH-region as a highly negatively charged ion, and therefore its presence in the diet has a negative impact on the bioavailability of divalent, and trivalent mineral ions such as Zn2+, Fe2+/3+, Ca2+, Mg2+ Mn2+, and Cu2+. Whether or not high levels of consumption of phytate-containing foods will result in mineral deficiency will depend on what else is being consumed. Moreover, phytates and oxalates usually form insoluble salts with mineral elements such as zinc, calcium and iron to prevent their utilization in the body (Sarkiyayi and Agar 2010). Some *garri* during processing are made with red oil and this may increase risk of heart attacks. Furthermore, regular intake of *garri* might cause ulcer due to the high levels of cyanide in the poor processed *garri*. This is consistent with past studies by kendirim *et al.,* (2009) soaking of *garri* resulted in a decrease in total cyanide content of 13-52% after 24 hours, 73-75% after 72 hours.

Using atomic absorption spectroscopy (AAS) heavy metals was determined, and the result varied: with cadmium (Cd) 0.73±0.09 ppm, lead (Pb) 0.61±0.35 ppm and asenic (As) 0.25±0.02 ppm had the highest concentration, whereas mercury (Hg) had the least concentration 0.19±0.02 ppm. This finding confirms similar by Saeed *et al.* (2021) reported that metals including copper, iron, zinc and manganese are known to have adverse biological effects on human health, at low level. These heavy metals, when ingested or absorbed by the body, can accumulate over time and lead to various health complications, including neurotoxicity, nephrotoxicity, hepatotoxicity, and carcinogenicity (Birniwa *et al.,* 2023). Heavy metal toxicity mostly encompasses damage to biomolecules such as lipids, proteins, nucleic acids (DNA and RNA) etc. They are similarly responsible for different human ailments including hepato-toxicity, neurotoxicity, heart disease, nephron-toxicity and geno-toxicity, cancers, and hypertensions (Singh and Sharma, 2020). Other common effects are neurological disorders, reproductive problems, immunological dysfunctions, and human developmental abnormalities (Jagaba *et al.,* 2020). The potential accumulation of heavy metals in food stuff from the soil or anthropogenic activities poses a significant concern for consumer safety (Jagaba, *et al.,* 2021). Heavy metals could be toxic and essential: Toxic heavy metals mainly Pb, Cd, Hg and as can cause metal poisoning to the patients. Some heavy metals are essential and required by the human body in trace amounts.

The bioaccumulation of heavy metals in food can be extremely dangerous for human health. These metals enter the human body via inhalation and ingestion. This contamination is determined by soil mobility and bioavailability (Yang *et al.,* 2015). Studies suggest that lead reduces the quality of tea and biomass by altering its chemical composition (Yongsheng *et al.,* 2011). It was found that exposure to even a low concentration of lead in plants cause extremely high ion instability, leading to large metabolic variation via potent inhibition of plant growth and photosynthetic capacity. Cadmium is the most important pollutant in the environment. Excessive and toxic levels of cadmium cause serious problems not only in humans but also in animals (Alina *et al.,* 2012). Cadmium concentrations in the blood below 10µg/L, suggest renal dysfunction (Bernard *et al.,* 2013). The risks of liver dysfunction are not widely described, although a single dose of cadmium can trigger toxicity. Chromium, a trivalent element, is an important mineral required for optimal health (Ghani, 2011). Chromium picolinate is used for the treatment of refractory type 2 diabetes mellitus, as it is absorbed comparatively better, than trivalent chromium. It also enhances insulin sensitivity. However, some studies showed that trivalent chromium exhibits toxicity (Welch *et al.,* 2016). The second form of chromium, hexavalent chromium, has been considered toxic in humans following chronic exposure orally or via inhalation with high levels causing damage to the kidneys, liver, and immune, blood and gastrointestinal systems. Ulceration and sensitivity of the skin, as well as contact dermatitis, can be caused by dermal exposure to this compound (Saha *et al.,* 2011). Numerous studies have established the increased levels of lead toxicity (Maksimiuk *et al.,* 2021). The concentration of Hg in urine is the most common biomarker of exposure to $Hg^0$ both in occupational exposures as well as to dental amalgams (Magos, 2000). Urinary mercury is derived from Hg that accumulates in kidney cells after acute (Reilly *et al.*, 2010). The concentration of Hg in blood is considered a reliable biomarker of exposure. In fish eating populations, metal levels in whole blood are usually interpreted a reflecting exposure to MeHg (Reilly *et al.*, 2010).

The total microbial counts obtained from the analysis of the *garri* samples showed that the total bacteria counts were higher than the fungal counts from the three sampling locations. This suggests a higher contamination by bacteria from the processors and the environment. The range for total bacterial counts $8.6 \times 10^{-7}$ CFU/g and total fungal counts $5.2 \times 10^{-4}$ CFU/g were within the tolerable limits of $10^4$ to $10^5$ recommended by the International Commission on Microbiological Specifications for Foods (ICMSF, 2010). The bacteria isolated in this study were *Escherichia coli*, *Micrococcus luteus, Streptococcus pneumonia* and *Staphylococcus aureus*. With *E.coli* had the highest frequency of isolation 136(40.2 %), followed by *M. luteus* 129(38.2 %), and *S. pneumonia* 39(11.5%), while the least frequency percentage of isolation 34(10.1 %) was recorded by *S. aureus*. It has been reported that around 10% of patients with invasive pneumococcal disease die from their illness (CDC, 2012). Among the range of pathogenic bacteria species that cause food borne diseases is *Escherichia coli*. The pathogenic strains of *Escherichia coli* cause diarrhea by producing and releasing toxins and can also be the cause *garri* spoilage. *Staphylococcus aureus*, a well-known commensal of human microbiota (Prescott *et al.*, 2002) can contaminate food process and handled with bare hands as such their presence in the *garri* samples suggest possible contamination from direct contact or aerial-droplet mechanisms such as coughing or sneezing by garri processors/retailers or handler (Prescott *et al.*, 2002). *Micrococcus luteus* is bacteria that can spoil *garri* and has been detected in the air, water, soil, plants, and food. The bacterium can contaminate foods such as garri, meat, fish, aquatic goods, soybean products, and other meals and has been identified in high concentrations in fresh, processed, and spoiled foods (Ding *et al.*, 2011). Humans infected with these bacteria can experience tissue inflammation, sepsis, shock, and other health hazards (Miltiadous *et al.*, 2012). *Streptococcus pneumoniae* can cause both minor infections, such as otitis and sinusitis, and severe invasive infections, such as community-acquired pneumonia and meningitis. This study revealed a generally poor sanitary state of the production, handling and retailing of *garri* which paved way for the contamination and proliferation of the microorganisms. Some of the bacteria encountered in this study are medically importance because they have been implicated in diverse human ailments (Prescott *et al.*, 2015).

The fungal genera isolated are; *Aspergillus flavus, Aspergillus niger, Aspergillus fumigatus, Aspergillus clavatus* and *Rhizopus sp*. were observed, with *A. flavus* had the highest frequency of isolation 10(38.5 %), followed by *A. niger* 8(30.8 %), *Aspergillus fumigatus* 3(11.5 %), and *Aspergillus clavatus* 3(11.5 %), while *Rhizopus sp* 2(7.7 %), had the least frequency of isolation. This is consistent with findings by Osho *et al.*, (2010), moulds such as *Aspergillus, Penicillium, Fusarium* have been associated with garri during storage and their growth results in changes in the organoleptic, microbiological and nutritive quality which lead to spoilage (Amadi and Adebola, 2008). Akano *et al.*, (2011) had reported that all the food contents of *garri* diminished following infection with these microbes. This, occur mostly due to the improper storage and lack of awareness, but most of them produce mycotoxins that are heat resistant and can cause food intoxication to consumers (Kabak *et al.*, 2012). The presence of these fungi in *garri* could be linked to poor handling and unhygienic spreading on of *garri* on the floor/ground/open basin in the markets for sale thereby giving room for the spores of these fungi which are ubiquitous in the surrounding to gain access to the commercial *garri*. This is consistent with past studies by Maren (2012) who reported that A. flavus spread yellow-green colonies and A. parasiticus dark yellow green 68 canidial areas. She further noted that bad smell and decaying could be due to activities of Aspergillus fungi which normally lead to garri spoilage and that members of Aspergillus genus are more heat tolerant and xerophilic than other fungal genera, therefore are very common garri spoilage organisms. This is consistent with findings by Lunyasunya *et al.* (2015) who reported that handling of mould infested food, may be harmful due to the presence of toxin and actinomycetes which are responsible not only for poisoning but also for allergy diseases affecting man known as „farmers lung" disease. The bacteria and fungi isolated from the *garri* samples in this research have been isolated and reported by other researchers such as Orji *et al.*, (2016); Akindele *et al.*, (2018); Okafor *et al.*, (2018). Their results validate the isolation of the microorganisms encountered in this study.

The amplified products obtained with primers specific for both *Escherichia coli* and *Aspergillus flavus* were 900-bp and 700-bp, respectively, which were the expected product sizes of the amplified gene with the set of primers used. This study is consistent with previous report of Turner (2005) who shows that ESBLs are mainly derived from TEM, SHV or CTX-M β-lactamases that have mutated to expand their spectrum of activity to include third generation cephalosporins. Although they were first reported in *Klebsiella species* (Kliebe *et al.*, 2006), ESBLs are now also commonly found in *Escherichia coli* and they have also been found in other species of *Enterobacteriaceae* (Bradford *et al.*, 2006). To date, more than 130 TEM and more than 104 SHV derivatives have been found (Bradford *et al.*, 2006). Merk *et al.*, (2006) found protienase K to be superior to other methods in extracting DNA. Other researchers have tried other synthetic lysing solutions like SDS (sodium dodecyl sulfate) and guanidine isothiocyanate (GITC) (Merk *et al*., 2006). TWEEN 20 (Dederich *et al*., 2007), Triton X-100 (Agersborg *et al*., 2009). GITC was reported to be able to damage cells with hard walls like fungi. Besides chemical methods, several researchers have successfully extracted bacterial DNA using physical power; for example, forceful rupture of cells was achieved by vortexing suspensions of cells (Marra *et al*., 2010), or beating cells with beads (Tell *et al*., 2012) or ultrasound waves (Picard *et al*., 2015).

However, present DNA purification procedures, especially the commercial ones, are costly, laborious and need a large number of reagents and equipment. Several researchers have tried to liberate DNA from bacterial cells by breaking bacterial cell walls using certain reagents, especially by enzymatic treatment with lysosymes and proteases (Porteous *et al*., 2021). However, Agersborg *et al*, (2009) reported that lysozyme and proteinase K treatment, was not always sufficienttohemolyse certain cells. Moreover, certain glass or iron beads were used to capture DNA molecules, which could later be eluted and separated (Skowronski *et al*., 2018). In fact, Tell *et al*., (2012) used cycles of freezing and thawing to obtain bacterial DNA. In practice, heating bacterial material for DNA extraction purposes was performed by boiling in a water bath or on hot blocks, or using microwave ovens (Porteous *et al*., 2021). In practice, heating bacterial material for DNA extraction purposes was performed by boiling in a water bath or on hot blocks, or using microwave ovens (Porteous *et al*., 2021).

## CONCLUSION

The *garri* samples contain nutritional factors, anti-nutritional, and heavy metals below the toxic level for human consumption. Bacteria and fungi detected are of public health relevance. DNA from *E. coli* and *A.flavus* are successfully used for PCR. Hence, these *garri* samples are good for human consumption and its compositions levels do not have any adverse effect on human health. Although in little quantity, these factors in the *garri* samples if taken for long period might cause some serious issues such as ulcer, eye defect, kidney disease and other ailments or diseases; therefore, people should avoid intake of *garri* regularly without proper thermal treatment so as to maintain disease/intoxication before consumption. There is need for the *garri* processors/retailers to maintain proper personal hygiene and safety in order to prevent food-borne infections or to reduce contamination by air-borne droplets and spores of the isolates in dust and air. Therefore, this study provides the following recommendations:

a. There is need for better *garri* storage facilities within markets and proper guidance must be provided by the regulating authorities for the safety and efficacy of *garri* during handling and storage.
b. The target populations are advice to minimize intake of *garri* without any form of thermal treatment so as to be protected from disease or intoxication.
c. Government should sponsor much research into more efficient way in order to isolate DNA and analyzed PCR for the rest of the isolated of both bacteria and fungi included in this research.

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