American Journal of
Food Sciences and Nutrition
(AJFSN)

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American Journal of Food Science and Nutrition
ISSN 2520-0461 (Online)
Vol.2, Issue 1, pp 32 – 44, 2020

www.ajpojournals.org

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Abstract

Purpose: This study was carried out with aim to determine the microbiological quality and safety of pupuru and garri on sale at Okitipupa main market in Okitipupa Township.

Methods: Pupuru and garri were analyzed to determine their microbial load and the level of aflatoxins contamination using microbiology standard protocols. A total of thirty samples each of pupuru and garri were collected from ten different points in Okitipupa main market for evaluation.

Findings: The results obtained showed that the bacterial load of pupuru and garri ranged from 2.1 to 7.6 x10^4 cfu/g while the fungi and yeast content ranged from 1.3 to 6.2 ×10^5 cfu/g. Also the level of aflatoxins contamination in pupuru and garri was at the range of 1.5µg/kg to 6.0µg/kg. The isolates of bacteria from all the market points are the following; P*seudomonas aeruginosa, Streptococcus faecalis, Lactobacillus species, Bacillus cereus, Escherichia coli, Staphylococcus aureus* and *Yersinia species.* While the isolated fungi include *Fusarium oxysporium, Aspergillus flavus, Aspergillus fumigatus, Saccharomyces cerevisae, Candida albicans, Penicillum species, Rhizopus stolonifera,* and *Mucor specie*s. Some potential pathogenic bacteria like *E. coli, Staphylococcus species a*nd *Bacillus specie*s were isolated from pupuru and garri, the minimum microbial load obtained and the level of aflatoxins contamination could not pose any health risk to the consumers.

Recommendation: There is need for the producers and the sellers of these products to be taught good processing procedures (GPP) as this will definitely improve microbiological quality and safety of the products.

Keys words: *Pupuru, Garri, Bacteria, Fungi, aflatoxins*

INTRODUCTION

Pupuru and garri are fine flours obtained from traditional fermentation of cassava. Both foods are popular meal among the people of Ikale tribe in Ondo State, Nigeria. They are derived from cassava tubers (*Manihot esculenta Crantz*). Cassava (*Manihot esculenta Crantz)* is a major root crop in the tropics and its starchy roots are significant sources of calories for more than 500 million people worldwide (Food and Agriculture Organisation, 1997). It is the most important root crop in Nigeria in terms of food security, employment creation, and income generation for crop-producing households (Jonathan, Bello, and Asemoloye, 2017). It supplies about 70% of the daily calories of over 50 million people in Nigeria (Longe, 1990). In 2017, Nigeria was the largest producer of cassava in the world (with about 59 million metric tonnes (International Institute of Tropical Agriculture, 2019). Currently, Pupuru is prepared, by peeling to remove the brownish thin outer covering to reveal the white fleshy inner portion. Which is submerged in large pot or bowl filled with water between four to five days. During this period the white fleshy tuber (white portion) become soft and Fermented.

The microorganisms involved in the submerged fermentation process include *B. subtilis, Klebsiella spp., C. tropicalis, C. krusei*, and a wide spectrum of latic acid bacteria (Adebayo, et al.,2013; Olusola, 1992; Tamang, et al., 2016; Okafor et al., 1984).The white portion is removed from the water and crushed with hand, the central pith and primary xylem provide some fibres is removed and discarded while the rest white portion(mash) is placed in clean sack bags or cloth bags for between eighteen and forty-eight hours to undergo more fermentation. The fermentation causes release of some bound minerals including calcium and magnesium (Hahn, 1989). The most important contribution of fermentation is the release from the plant tissue of the enzyme linamarase, which is involved in the breakdown of the linamarin and lotaustralin (cyanogenic glucosides) of cassava which release hydrogen cyanide and detoxifies the product (Okafor, 2007; Ikujenlola and Opawale, 2007). During the period of fermentation, the mash is dewatered by placing heavy objects on the sack bags or cloth bags.

At end of the fermentation period, the mash is sun-dried for about six hours and later heated in a flat iron pot with stirring. The white flour obtained is ground with a grinding machine to obtain fine powder (which called Pupuru powder) and packed into clean bag or close container for storage. While the preparation of garri includes the detaching of the root, washing, crushing, fermentation, removal of water and, roasting (Okafor, 2007). Pupuru and Garri are essential carbohydrate food with low protein and fat. Pupuru is prepared for meal by bringing desired volume of water to boil in a clean pot and pour desired volume of Pupuru powder into the boiling water. Stir and mix well until it is thick. The thick solid obtained is the Pupuru meal.

It can be served with any soup of your choice. Garri, a dry food is largely consumed by most people from Africa without cooking and can be consumed with the addition of sweeteners. However, poor production processing often results in microbial contamination of the products which could pose health risks to the consumers (Omosuli et al.,2017; Oranusi and Braide, 2012). For example, aflatoxins are genotoxic carcinogens and are the most toxic of the mycotoxins (Liu and Wu, 2010). They have been linked to liver cancer particularly in developing countries where implicated foods are known to contain high levels of aflatoxins. It is not possible to determine the threshold levels below which aflatoxins have no effect and therefore no tolerable daily intake

level has been recommended. However it is recommended that concentrations of aflatoxins in food should be reduced to the lowest levels reasonably achievable (Codex Alimentarius Commission, 2016**).**

2. MATERIALS AND METHOD

2.1. Samples Collection

Pupuru and garri samples were collected aseptically from the sellers at 10 different points in Okitipupa main market. Okitipupa main market was chosen as a study area because it is the major market for the supply chain of the products in Okitipupa local government. The samples after collection were labeled and taken to the Adekunle Ajasin University, Akungba-Akoko Microbiology laboratory for analysis which is usually not later than 12 hours after collection.

2.2. Microbiological Analysis The analysis involved total plate count, fungi count, and coliform count.

2.2.1. Isolation and Identification of Bacteria in Pupuru and Garri samples Samples of the pupuru and garri collected were serially diluted tenfold in which ten grams of each sample was diluted in 90 ml peptone water followed by homogenization by horizontal and vertical agitations for a few minutes to obtain 10^{-1} dilution. Further tenfold serial dilution was made up to 10^{-4} for colony count. 1 ml of volume of each dilution was spread plated in triplicate on de-Man Rogosa Sharpe Agar (MRS; Oxoid CM 361) and incubated anaerobically at 35°C for 48 h for the enumeration of lactic acid bacteria, and Plate Count Agar (PCA; Ovoid CM 325) incubated at 32°C for 48 hrs was used for the enumeration of aerobic bacteria. All plates were prepared in triplicate. The colonies were counted and recorded followed by isolation, purification, and storage on Nutrient Agar (Lab M) slants and kept at 4°C for further characterization and identification (Adetunji, et al.,2017; Holt,, 1994).

2.2.2. Isolation and Identification of Fungi in Pupuru and Garri samples

Samples of pupuru and garri collected were serially diluted tenfold in which ten grams of each of sample was diluted in 90 ml peptone water followed by homogenization by horizontal and vertical agitations for a few minutes to obtain 10^{-1} dilution. Further tenfold serial dilutions were made up to 10^{-5} for colony count 0.1 mL of each of the samples was also plated on potato dextrose agar (PDA) and cornmeal agar (CMA) supplemented with 60 µg per ml chloramphenicol for fungal isolation. This was incubated at 28°C for 5 days. All plates were prepared in triplicate. The colonies were counted and recorded (Adebayo, et al., 2013; Adetunji, et al., 2017).

2.3. Characterization of Isolates

Bacterial isolates were characterized and identified using series of cultural and biochemical tests such as Gram staining, indole reaction coagulase, oxidase and catalase. While fungi characterization and identification were done by using their morphology features on potato dextrose agar and cornmeal agar.

2.4. Determination the Aflatoxins level in Pupuru and Garri

The aflatoxins level in pupuru and garri were determined using enzyme linked immunosorbent Assay (ELISA). According to the Beacon manufacturer's instruction aflatoxin test kit. Sample Processing and Preparation: Fifty gram (50g) of each sample (pupuru and garri respectively) and 5.0g NaCl were transferred to a clean blender jar and 100ml of 80% methanol/water were added to the jar and blended for 1 minute in a high speed blender. The solutions were filtered through a filter paper (Whatman filter) and then 5ml of extract was diluted with 20ml of water, the mixture then were filtered through a glass fiber filter and the extracts were used for aflatoxins levels.

Determination of the Aflatoxins Levels in the Samples: The Reagents and samples extracts were allowed to reach room temperature prior to starting the test. The appropriate numbers of test wells were placed into a micro well holder and 50µl of enzyme conjugate were dispensing into each of the test well. Another 50µl of calibrators and sample extracts were added to the appropriate test wells by using clean pipette tip for each and also 50µl of antibody solution were added into each test. The plate were gently shaken to mix the contents and incubated for 10 minutes. The contents of the wells were washed into an appropriate waste container by overflow to fill the wells with laboratory quality distilled water and were repeated 4 times for a total of five washes (Atanda, 2005). Following the last washing, the wash solutions were removed by inverting the wells onto absorbent paper and 100µl of substrate was added into each well and shaken gently. Followed by incubation for 10 minutes, this was followed by addition of 10µl of stop solution into each well and shaking the plate rack gently to mix. The absorbance of the wells were read at 450nm using an ELISA reader and the printed results was interpreted quantitatively by graphing the absorbance of the calibrators (Y-axis, logq) versus the calibrator concentration (X-axis, logp) on logq/logp graph paper. A straight line was drawn through the calibrator points and the sample absorbance was located on the line. The corresponding point on the Y-axis is the aflatoxins concentration (µg/kg) of the sample (Leszcynska, et al., 2001).

2.5 Determination the pH of Pupuru and Garri

The pH was determined using Kent pH meter (Kent Ind. Measurement Ltd., UK) model 7020 equipment with a glass electrode as described by Adetunji, et al., 2017. Ten grams each of the pupuru and garri samples were weighed and dissolved in 100 mL sterile distilled water. The solutions were later decanted and the pH was measured.

2.6. Statistical Analysis

The Data analysis was done using SPSS v.20.0. Descriptive statistics were used to summarize sampling data. Independent sample t-test was used to calculate and compare between the bacterial and fungal loads from the various selling points. The odds ratio and difference in the prevalence of the potential pathogens were determined with Pearson's Correlation Coefficient. A value of less than 0.05 was read as significant. Odds ratio (OR) was used to measure the association between potential pathogens and factors such as samples mean of the total viable counts and the mean of aflatoxins content to separate the mean.

3. Results and Discussion

Table 1: Shows the mean values for the total colony count for bacterial isolates from pupuru and garri obtained from Okitipupa main market.

Sampling points	Total colony count of bacteria in Pupuru cfu/g x10^4	Total colony count of bacteria in Garri cfu/g x10^4
A	4.2	3.3
B	1.3	4.0
C	3.5	5.5
D	5.3	6.5
E	4.2	7.0
F	2.1	1.5
G	1.3	5.5
H	4.0	3.0
I	3.6	7.6
J	6.0	5.8

Results are presented as mean of triplicates samples

The bacterial load of pupuru and garri samples ranged from 1.3–to 7.0 ×10^4 cfu/g. The highest bacterial load was observed from Garri with microbial loads of 7.6 ×10^4cfu/g, while the lowest was observed from Pupuru with bacterial counts of 1.3 ×10^4 cfu/g (Table 1). The bacterial load observed during this study were of lower value when compared with the findings of Adebayo, et al., (2013), who reported a bacterial load of 8.1×10^6cfu/g from the fermented cassava flour sampled from the different market during their study. Among the organisms isolated were *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Lactobacillus species* and *Streptococcus faecalis.* It was observed that *Staphylococcus species* was isolated from all the selling points in the market, and similar trends have been reported earlier by researchers that had worked on fermented cassava products from Nigeria (Adetunji et al., 2017, Ogiehor, et al., 2007, and Olopade, et al., 2014). The presence of Staphylococcus spp. and Bacillus cereus in food are normally associated with individual hygiene of food handler. Bacillus cereus and Staphylococcus aureus are the major causes of food poisoning especially if found present at 10^5– 10^7 cfu/g (Centre for Disease and Control, 2019). Also presence of E. coli in some of the food samples may be hazardous to the consumer. It was reported by Nweze (2010) that E. coli was responsible for the causes of most food related diarrhoea. Meanwhile, samples from two selling points were found to have the highest amount of aerobic bacterial count of 7.6 × 10^4 cfu/g. These values were lower than the findings of microbial count of the fermented flour obtained by Adetunji et al., (2017) who reported 1.1 × 10^6 cfu/g. It was also lower than the findings of Ogiehor and Ikenebomeh (2005) who reported 2.7 × 10^3 to 1.2 × 10^7cfu/g.

Table 2: Shows the mean values for the total colony count for fungal isolates from pupuru and garri obtained from Okitipupa main market.

Sampling points	Colony count of fungai in Pupuru cfu/g x 10^5	Colony count of fungal in Garri cfu/g x 10^5
A	5.3	3.9
B	3.0	4.4
C	4.2	6.2
D	6.0	1.2
E	4.5	4.0
F	3.3	2.2
G	1.2	3.9
H	1.7	2.4
I	1.3	5.5
J	4.4	7.6

Results are presented as mean of triplicate samples

The fungi and yeast count found in pupuru and garri during this study were ranged between 1.2 to 7.6 x $10^{5.}$ This was a lower value when compared to the value of 8.2 x10^5 cfu/g observed by Adetunji, et al. (2017), from fermented cassava food products sold in Ilorin-west local government area, Nigeria. The isolated fungi included Fusarium oxysporium, Aspergillus fumigatus, Aspergillus flavus, Saccharomyces cerevisae, Candida albicans, Penicillum spp., Rhizopus stolonifera and Mucor spp.(Table 2). Some of the isolated fungi were identified to be spoilage inducing on foods during storage (Jonathan, Bello, and Asemoloye, 2017). The microbiological attributes of the raw cassava to be processed should be clean and of good quality. The usage of starter cultures that have antimicrobial properties with the capability to detoxify, in addition to sustaining adequate and clean surrounding is recommended. Also, sterilised packaging material should be used in packing the processed fermented food from cassava (Ikujenlola and Opawale 2007; Ogiehor and Ikenebomeh, 2005).The presence of fungi on various fermented cassava food products examined during this study might be due to the fact that the environmental condition favors their rate of sporulation (Olopade et al., 2014). Also, Oranusi and Braide (2012) reported in their study that some fungus species like Penicillium, Fusarium, and Aspergillus isolated from different samples during this study could produce poisonous substances which may be toxigenic when exposed to a favorable environmental condition. The occurrence of Aspergillus species found in most of the fermented cassava food products from all selling points in the market during this study might be due to the various methods used in the cassava processing. The same observations were reported by Adetunji, et al., (2017), Sanni, (1989), and Jonathan, Bello, and Asemoloye, (2017).

Table 3: Shows the mean values of the aflatoxins concentration for the various samples pupuru and garri obtained from the Okitipupa main market.

Sampling points	Aflatoxins in Pupuru µg/kg	Aflatoxins in Garri µg/kg
A	2.0	0.0
B	0.0	3.0
C	0.0	1.0
D	0.0	2.0
E	1.0	2.5
F	3.0	2.5
G	2.5	0.0
H	4.0	6.0
I	3.3	0.0
J	2.0	5.0

Results were presented as mean values of duplicate.

The aflatoxins contamination from samples of Pupuru and Garri tested ranged from 1.0µg/kg to 6.0µg/kg. These values were below the maximum acceptable limit of 10µg/kg stated by and suggested by National Agency for Food and Drugs Administration and Control (NAFDAC) in Nigeria (Atanda, 2005). The report is in agreement with that reported by researchers; Chiona, et al. (2014), Jonathan, Bello, and Asemoloye (2017), Kaaya and Eboku (2010), Okolie, et al. (2012), on the level aflatoxins contamination in cassava flour sold in some selected markets in Nigeria. It also agreed with Bankole, et al. (2006) who reported that cassava and yam were less susceptible to aflatoxins contamination. I observed that proper drying of pupuru and garri, and also kept them in an airtight container can prevent or minimize the level of contamination of these foods by aflatoxins, because three out of the ten selling points sampling that were properly dried and kept in airtight container were free from aflatoxins contamination.

Table 4: Shows mean values of the pH results obtained form pupuru and garri on sale in Okitipupa main market.

Sampling points	pH of Pupuru	pH of Garri
A	4.5	5.2
B	5.0	5.0
C	4.7	5.4
D	5.0	5.6
E	5.4	6.0
F	4.5	6.1
G	3.9	5.0
H	3.9	4.9
I	4.2	5.0
J	4.5	4.5

Results are presented as mean values of triplicate samples

The range of pH for pupuru and garri on sale in Okitipupa main market is between pH 3.9 to 6.7. It was observed that the result was comparable to the findings of Adebayo, et al. (2013) and Orji, et al. (2016). Meanwhile, the increase in pH observed in some selling points may be due to short period of fermentation by some of the processors thereby limiting the activities of lactic acid bacteria. This is because the product is always in high demand which allows some of these processors to compromise the quality of these products. And this may be hazardous to the consumers as observed by Capozzi, et al. (2017).

American Journal of Food Science and Nutrition
ISSN 2520-0461 (Online)
Vol.2, Issue 1, pp 32 – 44, 2020

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Table 5: Morphological and Biochemical Characteristics of the Bacterial Isolates from Pupuru and Garri Samples Purchased from Okitipupa Main Market in Ondo State, Nigeria.

Morphological and Biochemical Characteristics of the Bacterial Isolates							**Probable organisms**	
Morphology	**Biochemical Tests**							
	Gr	**Ox**	**Ca**	**Ind**	**Coa**		Pupuru	Garri
Circular, raised, shinny, smooth, colourless.	-	-	+	+	-		-	*Escherichia coli*
Oval shaped, clusters, creamy, raised, small colony with smooth surface	+	+	+	-	+		*Staphylococcus aureus*	*Staphylococcus aureus*
Rod shaped, blue-green pigment, small and mucoid colonies.	-	+	+	-	-		*Pseudomonas aeruginosa*	-
Coccobacillus, small, pink and shiny colonies.	-	-	+	-	+		*Yersinia* spp.	*Yersinia spp*
Rod shaped, dry, flat, and irregular.	+	+	+	-	-		*Bacillus cereus*	*Bacillus cereus*
Oval shaped in chains, colourless, dry, shiny or mucoid.	+	-	+	+	-		-	*Streptococcus* faecalis
Oval shaped, clusters, creamy, raised, small colony with smooth surface	+	+	+	-	+		*Staphylococcus aureus*	*Staphylococcus aureus*
Rod-shape,long and slender brick red coryneform cocobacillus.	+	-	-	+	-		*Lactobacillus species*	*Lactobacillus species*

Key: Gr= Gram reaction, Ox= Oxidase test, Ca= catalase test, Ind= Indole test, Coa= Coagulase test, + = Positive, - = Negative

Table 6: Morphology characteristics of fungi isolates from Pupuru and Garri as observed on PDA and CMA

Probable organisms	Description of isolates cultural and morphology features observed on PDA and CMA media
A. fumigatus	Colonies have dense felt of yellow-green conidiophores. Conidia heads typically radiate latter splitting in several loose columns, yellow-green becoming dark yellow-green. Sclerotia are brown to black.
A. flavus	Colonies have yellow-green conidiophores. Conidiophores have stipes with smooth-walled hyaline but often in brown colour.
F. oxysporium	Colonies have aerial mycelium with whitish or peach colour;Conidiophores are usually short branched on phialides.
Penicillum spp.	Colonies grow and sporulate with yellow or brown-green conidiophores with 3-6 phalides. Phalides often solitary, cylindrical with a short neck.
R. stolonifera	Colonies have whitish color becoming grayish-brownish. Sporangiophoresare colourless to dark brown, rough-walled stolons opposite the branched rhizoids. It has sporangia with sub-globose, ovoid, with blackish-brown color at maturity.
Muccor spp.	The colony was white and woolly. The hypha were thick and non-septate, columella were round. The sporagiopores departs laterally from mycelium, the sporangia were filled with spores.
S. cerevisiae	Colonies extent quickly and developed within three days. They have flat, moist, glittering or dull, and cream in color. Blastoconidia are present.
C. albicans	Colonies are whitish-cream in color, smooth, glabrous and yeast-like in appearance. Presence of spherical to sub spherical blastoconidia.

CONCLUSION

My results had shown that although pupuru and garri are fermented food products, there is possibility of contamination resulting due to poor handling either at the processing site or from the market. In the course of the investigation, it was observed that quality is often compromised in the displayed pattern in the market as it encourages cross-contamination.

RECOMMENDATIONS

It was suggested that the sellers in the market should use clean containers with cover to display their pupuru and garri. Also, the sellers should not display their pupuru and garri by the road sides so as to prevent contamination by aerosol microbes. In addition, the sellers should only bring pupuru and garri that are properly dried to sell in the market so as to prevent mycotoxins contamination. Furthermore, both the sellers and producers should be taught good production procedures (GPP) that ensure the safety and suitability of the food. Finally, the food handlers of these products need to be knowledgeable in food safety as to avoid food poisoning and food intoxication.

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