Microbiological and Nutritional Compositions of *Garri* Produced using Traditional Fermentation and Instant Mechanical Methods with and without added Palm Oil

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Abstract: *Garri* is a granular pre-gelatinized cassava starch with slightly fermented flavour and slightly sour taste made from grated, fermented fresh cassava tubers. It serves as staple food in many parts of Nigeria. This work studied the effect of fermentation and palm oil on the nutritional compositions of *garri* produced by traditional fermentation Method (TFM) and Instant Mechanical Method (IMM). Cassava tubers of TME 419 variety were used to produce *garri* using the two methods and with addition of palm oil to the bag before dewatering and the pot during toasting. For *garri* processed by TFM, the cassava tubers were peeled with knife, washed with tap water and grated. The cassava mash produced was allowed to stay for 24 hours in a bag before it was dewatered using heavy woods. It was allowed to ferment in the bag for 96 hrs and then toasted. For the *garri* produced by IMM, the cassava tubers were peeled with knife, washed with tap water and grated. The cassava mash produced was not allowed to ferment but was dewatered using heavy woods after grating and then toasted. The microbial load of the *garri* mash from both the TFM and IMM was determined by inoculating 0.1 ml aliquots of serially diluted cassava mash in triplicate to appropriate media. For the isolation of bacteria, 0.1 ml aliquots was inoculated by spread plate method on sterile Nutrient, MacConkey, *Salmonella-Shigella*, Mannitol Salt and and De Mann Rogosa Sharpe Agar plates and incubated for 48 hrs at 35^{o}C. For isolation of fungi, 0.1 ml aliquots were inoculated on Sabouraud Dextrose Agar (SDA) and incubated for 5 days at 22^{o}C. The colonies formed from both groups of isolates were sub-cultured on the same media and characterized through biochemical and sugar fermentation tests. The nutritional composition of the *garri* sample was determined using standard procedures. *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimuriun, Shigella dysentriae, Lactobacillus fermentum* and *Leuconostoc mesenteriodes* were the bacteria isolated from the grated cassava mash during fermentation while the fungal isolates are *Aspergillus niger, Fusarium solani, Penicillium notatum* and *Saccharomyce cerevisiae*. The highest bacterial load from the IMM (3.50 x 10^5 cfu/g) was from cassava mash without palm oil while the least (1.87 x 10^5 10^5 cfu/g) was from the mash mixed with palm oil before toasting. The highest bacterial load (2.60 x 10^5 CFU/g) from mashes produced using TFM was from the mash produced without palm oil while the lowest value (1.80 x 10^5 CFU/g) was from the mash to which palm oil was added during toasting. After fermentation, only *Bacillus, Lactobacillus* and *Leuconostoc* species were isolated while *S. cerevisiae* was the only fungus isolated. The *garri* produced from cassava that was fermented had a significantly higher carbohydrate and fats contents (87.29 and 3.07 respectively) than that produced by IMM. Addition of palm oil and the time of addition had no significant effect ($P<0.05$) on the protein, fibre and ash contents of the *garri* produced through fermentation. There was a significant reduction in hydrogen cyanide content of the *garri* samples produced through fermentation compared with the *garri* produced by IMM. In conclusion, the *garri* produced through fermentation has no pathogen/food spoilage organism in it. The hydrogen cyanide content was found to be reduced to tolerable limit and it has higher carbohydrate content.

Key words: Cassava tubers, food safety, *garri,* microorganisms, nutritional composition, traditional fermentation

INTRODUCTION

Garri is a granular pre-gelatinized cassava starch with a slightly fermented flavour and a slightly sour taste made from grated, fermented, gelatinized fresh cassava roots (Sokari and Karibo, 1992). This partially gelatinized dried cassava product is commonly consumed directly or soaked in cold water with sugar, coconut, roasted peanut, or boiled cowpea as compliments, or as a stiff gel made with hot water and eaten with soup or stew. The acceptance and popularity of *Garri* in urban and rural areas of West and Central Africa is attributed to its ability to store well, its convenience and ready-to-eat form (Flach, 1990). A safety concern among the consumers of cassava based products like *Garri* arises from the presence of cyanogenic glucoside, which upon hydrolysis produces cyanohydrin that further breaks down to release hydrogen cyanide- a known plant toxin (Bokanga 1994; Ernesto *et al*., 2002).

Garri is a staple food eaten mostly by the mid-western part of Nigeria as Red-*garri* and white *garri*. While the western part eats it as *Ijebu Garri*. There are several factors which influence the quality of *garri* namely processing conditions and storage conditions. Although all the processing steps are important to determining the end quality of *garri*, however, grating and fermentation remain the critical steps in *garri* processing. Hydrolysis is initiated by intimate contact between natural compartmentalized enzyme linamarase and linamarin (Vasconcelos *et al*., 1990), while fermentation is crucial to the development of the characteristic aroma, sour flavour of *Garri* and detoxification of the mash by liberation of free hydrogen cyanide (Achinewhu and Owuamanam, 2001).

The general traditional processing method of *garri*, has been found to be generally unhygienic and may cause serious health/environmental hazards to the final consumers. But very little has been done on the instant mechanical method of *garri* processing. Obadina *et al.* (2009) observed that after fermentation of the cassava product (*garri*) a change in odour was observed. This could be caused by the fermentation process involved, yielding unwanted organisms, therefore causing smell to the final products (Obadina *et al*., 2009).

Garri production till date is carried out at local levels mostly by local women who do so to enhance household food security (Fapojuwo, 2008), and according to Nweke *et al*. (2002), production of *garri* remains labour-intensive. Large scale production of *garri* in Nigeria failed probably due to the limited information on processing variables that promote detoxification in cassava, and fermentation to produce unique flavor characteristics associated with *garri* (Nweke *et al*., 2002).

Traditional processing of cassava by fermentation is centered on reduction of cyanide in the resultant product through extended period of fermentation for up to 7days as important strategy for the safety of product (Sanni, 2005). Very little attention has been paid to factors such as microbial load, nutritive value etc. However, even the traditionally processed *garri* contain varied amount of residual cyanide because of the tendency by local processors to shorten the duration of fermentation in order to meet growing market demand (Nweke *et al*., 2002). The fermentation of cassava has been reported to cause an increase in the protein and moisture content and a decrease in crude fiber, ash and carbohydrate (Obueh *et al.,* 2017).

Consumption of cassava and its products containing high amounts of cyanide and microbes can cause acute intoxication, with symptoms of dizziness, headache, nausea, vomiting, stomach pains, diarrhea and sometimes death (Oluwole *et al*., 2003). Very little study has been done in the area of *garri* processing using instant mechanical methods, which is why the present study is set to analyze the microbial and nutritive content of *garri* processed using traditional techniques and instant mechanical methods. The aim of this study was to determine the microbiological and nutritional compositions of *garri* produced using traditional fermentation and instant mechanical methods.

MATERIALS AND METHODS

Sample Collection

The cassava tubers used for this research work were sourced from the Cassava Research Program of National Root Crops Research Institute (NRCRI), Umudike and the botanical identity was confirmed as *Manihot esculanta* spp of variety TME 419. Laboratory facilities were from Ceslab Global Laboratory Services Umudike, Umuahia, Abia State.

Production of *garri*

For *garri* processed by traditional fermentation method, the cassava tubers were peeled with knife, washed with tap water and grated. The cassava mash produced was allowed to stay for 24 hours in a bag before it was dewatered using heavy woods. After, the *garri* was allowed to ferment in the bag for 96 hrs after which it was toasted in a big traditional pan (Olukosi *et al.,* 2017). Three *garri* samples were produced from the fermented *garri.* In the first one, the *garri* was fried without adding palm oil.

In the second *garri*, palm oil was added to the bag before fermentation while in the third sample, palm oil was added during toasting. For *garri* processed by instant mechanical method, the cassava was grated and dewatered using hydraulic press and fried without allowing it to undergo fermentation (Olukosi *et al.,* 2017). Three *garri* samples were produced from the instant mechanical method. In the first samples, the *garri* was fried without palm oil. The second one was *garri* with palm oil added in the bag after dewatering while in the third sample, palm oil was added to the *garri* during toasting. After the *garri* production, 500g of each *garri* sample was labelled and used for further analyses.

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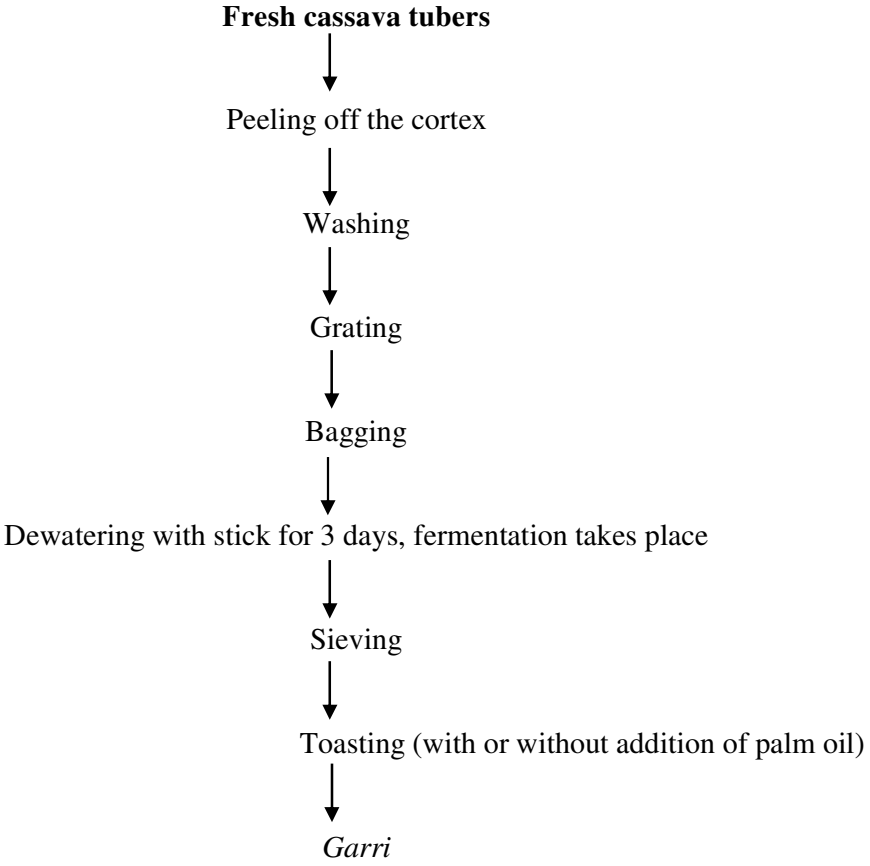

Figure 1: *Garri* processing by traditional fermentation method

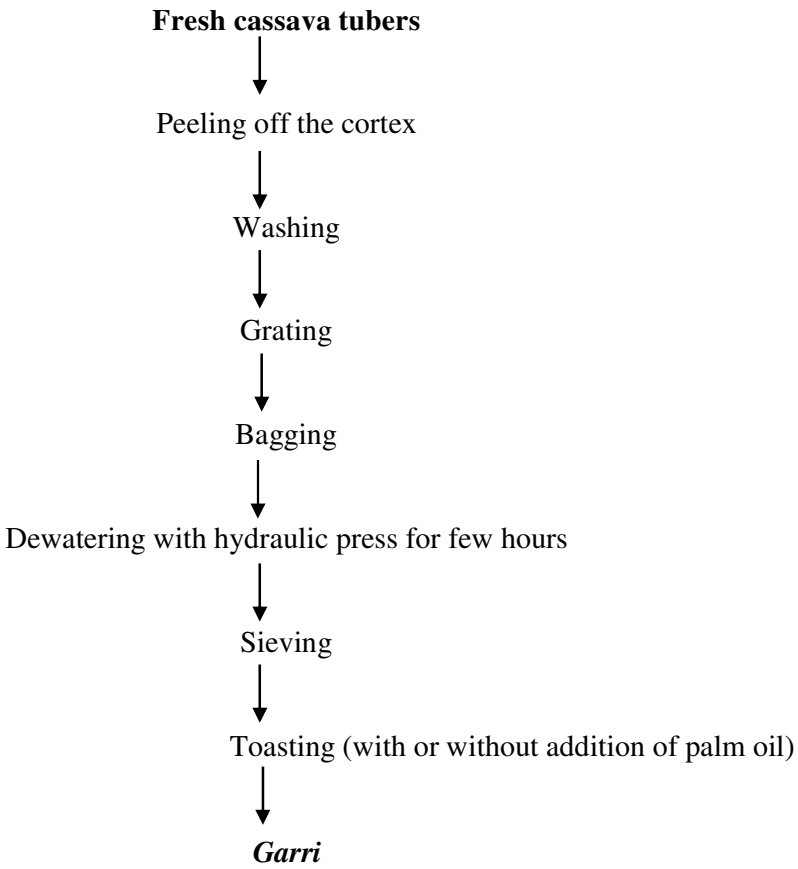

Figure 2: *Garri* processing by instant mechanical method

Three treatments were undertaking as follows:
Treatment 1: *Garri* produced without addition of oil to the instant and the fermented replicates.
Treatment 2: *Garri* produced with the addition of oil in the bag prior to dewatering and in both the instant and the fermented.
Treatment 3: *Garri* produced with the addition of oil in the toasting pot in both the instant and fermented *garri.*
T_1 = No oil added, T_2 = Oil added in the bag, T_3 = Oil added during toasting, R_1 = Instant *garri*
R_2 = Fermented *garri*

Determination of Microbial Load of cassava mash

The microbial loads of the cassava mash from both the traditional fermentation and instant mechanical methods were determined. One gram of each of the cassava mashes was serially diluted in 9ml of sterile peptone water in test tubes and 0.1 ml aliquots was inoculated to appropriate media for microbial isolation. This was done in triplicate. For the isolation of bacteria, 0.1 ml aliquots was inoculated by spread plate method on sterile Nutrient, MacConkey, *Salmonella-Shigella*, Mannitol Salt and De Mann Rogosa Sharpe Agars and incubated for 48 hrs. at 35^{o}C. For isolation of fungi, 0.1 ml aliquots was inoculated on Sabouraud Dextrose Agar (SDA) for 5 days at 22^{o}C. The colonies formed from both groups of isolates were sub-cultured on the same media they were inoculated initially and re-incubated at the conditions mentioned earlier. The pure colonies were stored in culture slants and kept in refrigerator at 4^{o}C for further use (Cheesbrough, 2005).

Microbial Succession

This was carried out for both the traditional fermentation and Instant Mechanical processing methods.

One gram of the mash was aseptically collected from each of the *garri* processing mashes and serially diluted as stated earlier in 9 ml of sterile peptone water in test tubes. 0.1 ml aliquots of appropriate dilutions was inoculated onto sterile plates of Nutrient Agar and SDA and incubated at appropriate conditions. The isolates were sub-cultured and the pure isolates were stored in agar slants in refrigerator at 4^{o}C till further use. This test was done every 24 hours till the fermentation process was over.

Characterization of Bacterial Isolates

The bacterial isolates were characterized through morphological, Gram Staining and biochemical tests which include catalase, oxidase, coagulase and urease tests. Others are nitrate reduction, indole production, utilization of nitrate, the methyl red, Voges Proskeur tests and sugar fermentation tests (Cowan and Steel, 1965).

Characterization of Bacterial Isolates

Characterization of fungi isolate was based on a study of their general and peculiar characteristics at the cultural (plate) level and at the structural (microscopic) level. Each fungi isolate was set up in a slide mount and stained with Lactophenol cotton blue dye solution and thereafter examined under the microscope using the x40 objective lense. All observed structural features were recorded including the directional growth of the sporangiophores or conidiophores, the presence of branchings, septation as well as the term of the apex head. The shape of fruiting bodies like spores and conidia was observed and recorded. All the recorded characteristics of the fungi isolate were reserved for cross-matching with existing information in standard mammal for identification (Barnett and Hunters, 1987).

Determination of Nutrient Composition of the *garri* Samples

The proximate analysis of the samples were carried out according to the methods outlined by the Association of Official Analytical Chemists (A.O.A.C, 2000).

Determination of Moisture content

Two grams (2g) of each sample was weighed into a crucible. The crucible and its sample content were dried in the oven at 105^{o}C for 3 hours. It was cooled in a desicator and reweighed. The weight was recorded while the sample was returned to the oven for further drying. The drying, cooling and weighing was repeatedly done until a constant weight was obtained.

% moisture content was calculated as;

$$\% \text{ moisture} = \frac{W_2 - W_3}{W_2 - W_1} \times \frac{100}{1}$$

Where:

W_1 = weight of dried crucible

W_2 = weight of crucible + sample before drying

W_3 = weight of crucible + sample after drying to a constant weight.

Determination of Protein content

Five grams (5g) of the sample was mixed with 10ml of concentrated sulphuric acid in a Kjedahl digestion flask. A tablet of selenium catalyst was added to it and the mixture was digested under a fume cupboard until a clear solution was obtained. In a separate flask, the acid and other reagent were digested but without the sample to form the blank control. The entire digest were carefully transferred to a 200ml/vol flask using distilled water and made up to a mark in the flask. 10ml portion of each digest was mixed with equal volume of 45% NaOH solution in Kjedahl distilling unit. The mixture collected into 10ml of 4% boric acid solution containing 2 drops of mixed indicator. A total of 50ml distillate was obtained and titrated against 0.02M H_2SO_4 solution.

The nitrogen content was calculated as shown below;

$$\%N_2 = \frac{(100\ x\ n\ x\ 14\ VF)\ \ T}{W\ x\ 100\ x\ VA}$$

Where
W = weight of sample analyzed (g) , n = concentration of the conc. H_2SO_4 , VF = total volume of digest (m^3) , VA = volume of digest distilled (m^3) , T = titre value blank , % crude protein = $\%N_2$ x 6.25 , Where 6.25 is the conversion factor.

Determination of Fat content

Two grams (2g) of the sample was wrapped in a Whatman No.1 filter paper. The wrapped paper was put in a Soxhlet flask containing 200ml of ethanol. The upper end of the reflux flask was connected to a condenser. By heating the solvent in the flask through electro thermal heater, it vaporizes and condensed into the reflux flask. Soon the wrapped sample was completely immersed in a solvent and remains in contact with it until the flask filled up and siphoned over thus carrying oil with it from the sample down to the boiling flask. The process was allowed on repeatedly for 4 hours after which the sample was removed and reserved for crude fibre analysis. The solvent was recorded and the extraction flask with its oil content was dried in the oven at 60^{o}C for 30minutes (i.e. to remove any residual solvent). After cooling in a desiccator, the flask was rewashed.

The weight of the oil extracted was determined and expressed as a percentage of the sample weight;

$$\% \text{ fat} = \frac{W_2 - W_1}{W_3} \times \frac{100}{1}$$

Where
W_2 = weight of flask + oil
W_1 = weight of empty flask
W_3 = weight of sample used

Determination of Crude fibre

Two grams (2g) of the sample was defatted during fat analysis. The defatted sample was boiled in 200ml of 1.25% H_2SO_4 solution under reflux for 15 minutes. The sample was washed with several portions of hot (boiling) water using a two-fold muslin cloth to trap the particles (residue). The residue was carefully transferred back to the flask and 200ml of 1.25% NaOH solution was added to it. Again the sample was boiled for 15 minutes and washed as before with hot water. Then it was transferred to a weighed porcelain crucible and dried in the oven at 105^{o}C for 3 hours. After cooling in a desiccator, it was weighed and then put in a muffle furnace and burnt at 550^{o}C for 2 hour until it became ash. Again, it was cooled in a desiccator and reweighed.

$$\% \text{ crude fibre} = \frac{W_2 - W_3}{W_1} \times \frac{100}{1}$$

Where
W_2 = weight of crucible + sample after washing
W_3 = weight of crucible + sample as ash

Determination of Carbohydrate content

Carbohydrate content was determined by the difference method. This was done by summing up the % moisture, % protein, % fat, % ash and % crude fibre contents and then subtracting their sum from 100. It was also expressed in percentage (%).

Statistical Analysis

Obtained data from the analysis were subjected to statistical Analysis of Variance (ANOVA) to determine the level of variations. The Statistical Package for Social Sciences (SPSS) Version 20 software was used for the work.

RESULTS

Table 1 shows the bacterial isolates from the cassava mashes**.** They include *Escherichia coli***,** *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimuriun, Shigella dysentriae, Lactobacillus fermentum* and *Leuconostoc mesenteriodes.* The fungal isolates from the grated cassava mashes are indicated on Table 2 and they include *Aspergillus niger, Fusarium solani* and *Penicillium notatum.* Table 3 shows the microbial load of the cassava mashes from traditional fermentation and instant mechanical methods with and without addition of palm oil addition at different stages of the production processes. The highest bacterial load for the instant mechanical method was 3.50 x 10^5 10^5cfu/g from the mash without palm oil while the least (1.87 x 10^5 10^5 cfu/g) was from the mash mixed with palm oil before toasting.

For the mashes produced using traditional fermentation method, the highest bacterial load (2.60 10^5 x cfu/g) was from the mash produced without palm oil while the lowest value (1.80 10^5 x cfu/g) was from the mash to which palm oil was added before toasting. For the mash produced from instant mechanical method, the highest fungal load (7.67 x cfu/g) was from the sample produced without palm oil while the least value (3.33 x cfu/g) was from the sample to which palm oil was added during toasting. For the mashes samples produced using the traditional fermentation method, the highest fungal load (5.67 x cfu/g) was from the mash without palm oil while the lowest value (2.67 x cfu/g) was from the mash mixed with palm oil during toasting.

The result of bacterial succession in the cassava mashes with and without addition of palm oil is presented in Table 4. It shows that seven bacterial general namely *Staphylococcus, Escherichia, Bacillus, Lactobacilus, Leuconostoc, Shigella* and *Pseudomonas* participated in the fermentation of the cassava mash without palm oil. All of them were present at the beginning of the fermentation, but at the end of the fermentation only three genera namely *Bacillus, Lactobacillus* and *Leuconostoc* species were isolated.

In the mash fermented with palm oil, seven bacterial general namely *Staphylococcus, Escherichia, Bacillus, Lactobacillus, Leuconostoc, Shigella* and *Pseudomonas* species were isolated during the course of the fermentation. At the end of the fermentation, only *Bacillus, Lactobacillus* and *Leuconostoc* species were recovered from the mash.

The result of fungal succession in the cassava mashes with and without addition of palm oil is presented in Table 5. *Saccharomyces*, *Aspergillus, Penicillium* and *Fusarium* species were isolated from the mashes with and without palm oil. At the end of the fermentation, only *Saccharomyce cerevisiae* was isolated from the two types of mashes.

Table 6 shows the nutritional composition of the *garri* produced by traditional fermentation and instant mechanical methods. From the results, there were significant variations in the proximate (major nutrient) composition of the products. The moisture content varied between 6.46% and 7.09% and was higher in non-oil *garri* samples. The protein content was in the range of 2.22% to 2.44% and did not show any significant difference irrespective of the type of *garri* (instant or fermented, oil or no-oil). The fat content was higher in the oil samples than in the non-oil samples with a range of 1.50% (instant *garri* without oil) to 3.07% (instant *garri* with oil added in the pot). The fat content of the oil *garri* samples was 2.45% and 3.00% for the instant *garri* with oil added in bag and in the pot respectively, while the fermented oil *garri* recorded fat contents of 2.21% and 3.07% for the ones in which oil was added in the bag prior to fermentation and the one oil was added in the pot during toasting respectively. The fibre content was the same 1.89% in the *garri* without oil both instant and fermented but was slightly but insignificantly lower in the oil *garri* added to the pot (1.85% to 1.86%).

Ash content did not show much significant variations but was higher in the oil *garri* samples with a range of 1.43% to 1.46% as against 1.36% to 1.37% recorded for non-oil *garri* samples.

The carbohydrate content was very high in all the *garri* samples irrespective of the type. The range was between 84.71% and 85.79% and was slightly but significantly higher in the non-oil *garri* samples. The carbohydrate content was 85.71% and 85.79% in the fermented and instant *garri* with oil respectively but recorded 85.29% and 85.51% in the fermented and instant *garri* with oil added in the bag respectively. The *garri* with oil added in the pot during toasting had carbohydrate values of 84.71% and 84.79% for the fermented and instant *garri* respectively.

The results in general showed variations in the proximate (nutritional) composition of the *garri* produced by the two different methods

Table 1: **Morphological and Biochemical Characteristics of Bacterial Isolates from fermenting cassava mash**

S/N	Colony appearance	Microscopic shape	Gram reactions	Catalase test	Coagulate test	Oxidase test	Indole test	Citrate test	Glucose	Lactose	Sucrose	Organism isolated
1	Pinkish circular rough colonies	Rod	-	-	-	-	+	-	+AG	+AG	-	*Escherichia coli*
2	Yellow circular smooth colonies	Cocci	+	+	+	-	-	-	+A	+A	+A	*Staphylococcus. aureus*
3	Pale coloured colonies	Rod	-	+	-	+	-	+	+A	-	-	*Pseudomonas aeruginosa*
4	Irregular creamy flat colonies	Rod in chains	+	+	+	-	+	+	+A	+A	+A	*Bacillus subtilis*
5	Transparent colonies with black center	Short rods	-	-	-	-	-	+	+AG	-	-	*Salmonella typhimuriun*
6	Transparent colonies with entire margin	Slender rods	-	-	-	-	-	-	+AG	-	-	*Shigella dysentriae*
7	Yellow colonies	Cocci	+	-	-	-	-	-	+	+	+	*Lactobacillus fermentum*
8	Flat dull wax entire colonies as white or opaque	Round colonies in pairs or clumps	+	-	-	+	-	-	+A	+AG	+AG	*Leuconostoc mesenteriodes*

Key: + = Positive, - = Negative, AG = Acid and gas production, A= Acid production only

Table 2: Fungal isolates from fermenting cassava mash

S/n	Surface	Elevation	Colour of spore	Appearance of mycelium	Reproductive structure	Septation	Probable Fungal isolate
1	Powdery	Raised	Black	Thread-like	Conidiospore	Branched	*Aspergillus niger*
2	Cottony	Raised	White	Aerial-mycellium	Macro-conidia & micro conidia	Branched	*Fusarium solani*
3	Powdery	Raised	Blue-green	Multi nucleated	Conidiospore	Branched	*Penicillium notatum*

Table 3: Microbial Load of Cassava mashes produced by Traditional Fermentation and Instant Mechanical Methods

Sample	Bacterial load (cfu/g) x 10^5	Fungal load (cfu/g) x 10^3
Instant Mash without Palm Oil	3.50^{a}±3.61	7.67^{a}±0.58
Instant Mash with Palm Oil added in the bag	3.33^{a}±1.16	5.33^{b}±1.53
Instant Mash mixed with Palm Oil during toasting	1.87^{c}±2.08	3.33^{c}±0.58
Fermented Mash without Palm Oil	2.60^{b}±2.01	5.67^{b}±1.53
Fermented Mash mixed with Palm Oil in the bag	2.47^{b}±2.51	4.67^{bc}±0.58
Fermented Mash mixed with Palm Oil during toasting	1.80^{c}±3.00	2.67^{d}±0.58

Values are means of triplicate analysis ± standard deviation. Values with different superscripts in the same column are significantly different ($p \leq 0.05$).

Table 4: Bacterial Succession in Fermenting Cassava Mash (hr)

	Samples	Time of fermentation				
		0	**24**	**48**	**72**	**96**
1	Fermented *garri* without oil	*Lactobacillus fermentum*	*Lactobacillus fermentum*	*Lactobacillus fermentum*	*Lactobacillus fermentum*	*Lactobacillus fermentum*
		Bacillus subtilis	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*
		Staphylococcus aureus *Escherichia coli*	*Staphylococcus aureus* *Escherichia coli*	*Staphylococcus aureus* *Escherichia coli*		
		Leuconostoc mesenteroids	*Leuconostoc mesenteroids*	*Leuconostoc mesenteroids*	*Leuconostoc mesenteroids*	*Leuconostoc mesenteroids*
		Shigella dysentriae	*Shigella dysentriae*	*Shigella dysentriae*		
		Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*		
2	Fermented *garri* with oil in the bag	*Lactobacillus fermentum*	*Lactobacillus fermentum*	*Lactobacillus fermentum*	*Lactobacillus fermentum*	*Lactobacillus fermentum*
		Bacillus subtilis	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*
		Staphylococcus aureus	*Staphylococcus aureus*	*Staphylococcus aureus*		
		Escherichia coli *Leuconostoc mesenteroids*	*Escherichia coli* *Leuconostoc mesenteroids*	*Escherichia coli* *Leuconostoc mesenteroids*	*Leuconostoc mesenteroids*	
		Shigella dysentriae	*Shigella dysentriae*			
		Pseudomonas aeruginosa	*Pseudomonas aeruginosa*	*Pseudomonas aeruginosa*		

Table 5: Fungal Succession in Fermenting Cassava Mash (hr)

	Samples	0	24	48	72	96
1	*Garri* Fermented without Palm oil	*Aspergillus niger* *Fusarium solani* *Penicillium notatum* *Saccharomyces cerevisiae*	*Aspergillus niger* *Fusarium solani* *Penicillium notatum* *Saccharomyces cerevisiae*	*Aspergillus niger* *Fusarium solani* *Penicillium notatum* *Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
2	*Garri* Fermented with Palm oil in the bag	*Aspergillus niger* *Fusarium solani* *Penicillium notatum* *Saccharomyces cerevisiae*	*Aspergillus niger* *Fusarium solani* *Penicillium notatum* *Saccharomyces cerevisiae*	*Aspergillus niger* *Fusarium solani* *Penicillium notatum* *Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*

Table 6: Nutritional Content of *Garri* Produced Using Traditional Method and Instant Mechanical Methods (%)

Sample	Moisture content	Protein content	Fat content	Fiber content	Ash content	Carbohydrate	Hydrogen Cyanide content
Instant *garri* without Palm oil	7.06^{a}±0.10	2.39^{a}±0.10	1.50^{d}±0.03	1.89^{a}±0.05	1.37^{b}±0.04	85.79^{a}±0.33	9.03±0.01^{d}
Instant *garri* with Palm oil in the bag	6.91^{b}±0.03	2.25^{a}±0.05	2.43^{b}±0.05	1.89^{a}±0.05	1.43^{a}±0.01	85.10^{a}±0.05	9.00±0.01^{d}
Instant *garri* with Palm oil in the pot	6.49^{c}±0.03	2.42^{a}±0.31	3.00^{a}±0.10	1.86^{a}±0.02	1.45^{a}±0.02	84.79^{a}±0.34	9.00±0.01^{d}
Garri Fermented without Palm oil	7.09^{a}±0.02	2.22^{a}±0.05	1.50^{d}±0.03	1.88^{a}±0.05	1.36^{b}±0.02	87.71^{a}±0.03	5.44±0.01^{c}
Garri Fermented with Palm oil in the bag	6.91^{b}±0.03	2.25^{a}±0.05	2.21^{c}±0.06	2.89^{b}±0.23	2.15^{b}±0.06	87.29^{a}±0.16	5.11±0.01^{b}
Fermented *garri* with Palm oil in the pot	6.46^{c}±0.02	2.44^{a}±0.29	3.07^{a}±0.02	1.85^{a}±0.01	1.46^{a}±0.02	74.71^{c}±0.33	5.22±0.03^{a}

Values shows means of triplicate analysis of each sample ± standard deviation. Figures with different superscripts in the column are significantly different

DISCUSSION

The determination of the nutritional compositions of *garri* produced using traditional fermentation and instant mechanical methods with and without the addition of palm oil was carried out in this work. Eight bacterial isolates were isolated from the fermenting cassava mashes and they include *Escherichia coli*, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus subtilis, Salmonella typhimuriun, Shigella dysentriae, Lactobacillus fermentum* and *Leuconostoc mesenteriodes.* They occurred equally both in the mash with and without added palm oil. The fermentation of cassava is mediated by a mixed bacterial consortium which ensures proper hydrolysis of the various nutrients present in the cassava tuber.

The isolation of the following fungi: *Aspergillus niger, Fusarium solani* and *Penicillium notatum* and the yeast: *Saccharomyces cerevisiae* from the fermenting cassava mashes shows the position occupied by these fungi in the breakdown of cassava tuber during fermentation.

Bacillus spp. yeast, filamentous fungi and Lactic acid bacteria have been reported in traditional fermented cassava (*lafun*) (Oyewole and Odunfa, 1989). This too agrees with the findings of Obadina *et al.* (2007) in which they isolated similar fungi from 'fufu' flour stored at different relative humidity in ambient condition.

The bacterial load and fungi load were found to be within tolerable level and though they are relatively high. The bacterial load were all below 1.0 x 10^6cfu/g which is the unacceptable level (Akindele and Abimbola, 2018). Similarly, the fungi load were within tolerable level as they were less than 1.0 x 10^4cfu/g. Though, the relatively high microbial load shows that there was high level of microbial consortium involved in the fermentation as starter cultures.

The bacteria flora and succession during fermentation show the presence of diverse species of bacteria. At the onset of the *garri* production, the cassava mash contained many different bacteria type including Gram positive (*Lactobacillus, Staphylococcus, Bacillus, Leuconostoc*) and Gram negative (*Pseudomonas, E. coli, Shigella* and *Salmonella*) bacteria. At the end of the fermentation (after 96 hours), only *Bacillus, Lactobacillus* and *Leuconostoc* were isolated from the fermented cassava mash. The elimination of pathogens such as *S. aureus* from the fermented cassava mash is of public health importance because it has been reported that *S. aureus* in *garri* may lead to possible intoxication since it produces harmful toxins (Akindele and Abimbola, 2018). So fermentation makes the *garri* safe for consumption. *Lactobacillus* spp does not only produce organic acids in fermented foods, they also contribute positively to the extension shelf-life of the food product by eliminating food borne spoilage organisms.

At the end of the fermentation, only *Saccharomyces cerevisiae* was isolated among all the fungi involved in the fermentation process. The elimination of *Aspergillus niger* (which produces Aflatoxin, a powerful mycotoxin) and *Fusarium solani* and *Penicillium notatum* which cause food spoilage is a proof that fermentation among other things eliminates pathogens due to the various metabolites produced by other microorganisms during fermentation. This ensures food safety.

The *garri* produced from cassava that was fermented was found to have a significantly higher nutritional composition than that produced by instant mechanical method. The *garri* produced from the mash which had palm oil added to it in the pot had the highest carbohydrate composition than the other *garri* samples. This could mean that the palm oil actually inhibited starch hydrolysis to simple sugars which would have been used by the microorganisms for their metabolic activities and a resultant loss in carbohydrate content. However, the high carbohydrate value of the *garri* produced through fermentation makes it a high energy giving food.

There was a significant reduction in hydrogen cyanide content of the *garri* samples produced through fermentation. The content dropped drastically below 10 mg HCN eq/mg, the World Health Organization (WHO) safe level of 1 mg/100 g (FOA/WHO, 1991).

However, the hydrogen cyanide contents of the *garri* samples produced though instant mechanical methods had statistically the same level of hydrogen cyanide levels. Lactic acid bacteria are known to cause the degradation of the cyanogenic glycosides such as amygdalin, linamarin, and linseed cyanogensm leading to a decrease in the concentration to levels that are non-toxic to the body (Lei *et al.,* 1999). Consumption of food containing hydrogen cyanide can cause acute intoxication, with symptoms of dizziness, headache, nausea, vomiting, stomach pains, diarrhea and sometimes death (Oluwole *et al*., 2003).

Our results confirm those obtained by Agbor-Egbe *et al.* (1995) confirming fermentation is then a very effective process for elimination of endogenous cyanic compounds from cassava tubers. The inhibitive effect of the cyanide on the lactic acid bacteria is weak because these bacteria tolerate high concentrations of cyanide (800 ppm; Louembe *et al*., 1997), while the growth of the other bacteria, such as *E. coli,* is totally inhibited by a cyanide concentration of 2 to 3 ppm (Knowles, 1976). Giraud (1993) reports that the growth of lactic bacteria strains is inhibited by concentrations of cyanide close to 1000 ppm. This resistance property is responsible for the dominance of lactic acid bacteria in natural microflora of cassava retting. It shows that these microorganisms are adapted well to the contents of cyanide present in cassava-retting roots. Vasconcelos *et al.* (1990) observed that the degradation of cyanogenic compounds during the fermentation of cassava, leads to the accumulation of free cyanide, which can reach 200 ppm in the fermenting medium.

Addition of palm oil to the *garri* samples during production by fermentation process reduced the hydrogen cyanide contents of the *garri.* This implies that it is not healthy and advisable for people to produce and eat *garri* using the instant mechanical method as the hydrogen cyanide in *garri* remains in the product. Cassava tubers are potentially toxic due to the presence of cyanogenic glycosides, linamarin and a small amount of lotaustralin which are catalytically hydrolyzed to release toxic hydrogen cyanide (HCN) when the plant tissue is crushed (Seri *et al.,* 2013). So, most processing techniques have been developed in different parts of the world to reduce the HCN content to an acceptable level (Etsuyankpa *et al.,* 2015). Results obtained from this study are in agreement with previous workers who found that *garri* produced from cassava tubers with palm oil has lower cyanide content than samples without palm oil (Fomuyan *et al*., 1981; Agbor, 2005, Odoemelam, 2005). This reduction may be due to the facilitation of volatilization of cyanohydrin and hydrogen cyanide by the palm oil thus making drying process easier and quicker during toasting (Vasconcelos *et al*., 1990).

The increase in fat was slightly significantly in the *garri* produced with palm oil during fermentation. This increase in fats was believed to be from the palm oil added to the fermenting mash. The increased fat content is desirable in foods as fat helps to improve flavor retention, insulate the body as well as act as solvent for fat soluble vitamins (Vitamin A, D, E and K) (Oluwaseun *et al.,* 2014). The result also indicates that the addition of oil and the time of addition had no significant effect ($p<0.05$) on the protein, fibre and ash contents of the *garri* produced through fermentation.

CONCLUSION

This work has given support to the report that fermentation reduces the cyanide content of cassava and *garri* produced from it. However, the cyanide content of *garri* produced by instant mechanical method remains unchanged thus posing a health challenge to those who consume the *garri* produced through that method. It also shows that pathogens and food spoilage microorganisms are eliminated from cassava and by extension the *garri* during fermentation. By this, *garri* is made safe for consumption and its shelf life extended too. Addition of palm oil to the cassava mash during fermentation improves the carbohydrate content of the *garri.*

RECOMMENDATION

From our findings here, we recommend that traditional method of fermentation of cassava mash be adopted over and above the instant mechanical method in *garri* production. We also recommend that addition of palm oil to fermented cassava mash during *garri* production.

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