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Exploring the Influence of Locations on Cyanide, Moisture and Microbial Loads of Gari Produced from five Major Processing Areas in Abia State, Nigeria

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***Abstract* — This study investigated the cyanide, moisture and microbial content of white and yellow *gari* sampled from the five market locations using standard analytical methods. Cyanide levels of white *gari* samples from Ndoki market location was least (0.122mgHCN) and highest (0.429mgHCN) in Isialangwa market location. Yellow *gari* samples had the least cyanide content (0.121mgHCN) in Ndoki market location and highest (0.373mgHCN) in Isialangwa market location. Moisture content of white *gari* samples was least (6.32%) in Ndoki market location and highest (10.16%) in Ndoro market location while yellow *gari* was least (5.82%) in Uzuakili market location and highest (11.26%) in Ariara market location. Total bacterial count of white *gari* was least (2.15cfu/g) in Ndoki market location and highest (3.10cfu/g) in Ariara market location. Yellow *gari* sample was least (2.40cfu/g) in Ndoki market location and highest (3.20cfu/g) in Ariara market location. Total fungi count of white *gari* was least (1.16cfu/g) in Ndoki market location and highest (2.70cfu/g) in Ariara market location while the yellow *gari* cyanide was least (1.07cfu/g) in Ndoki market location and highest (2.90cfu/g) in Ariara market location. All the quality indexes evaluated varied significantly (p<005) with market locations and lower than recommended safe limits.**

Keywords* — *location; hydrogen cyanide, gari, cassava, microbial load

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I. INTRODUCTION

Cassava, (*Manihot esculenta crantz*) is a widely grown dicotyledonous root crop in tropical regions of Africa, Latin American and Asia. Cassava roots are rich in carbohydrates mainly starch, major energy source, contains calcium, phosphorous, some essential vitamins and good source of dietary fiber [1]. Cassava roots have an average composition of 60 to 65% moisture 40% to 45% carbohydrates, 0.2 to 0.6% crude fat, 1 to 2% crude protein and low content of some vitamins and minerals [2]. Cassava roots contain cyanogenic glucosides (linamarin (95%) and lotaustralin (5%)) in all parts of the mature cassava plant which are potentially toxic [3]. Lotaustralin (ethyl linamarin) is located in the plant cell vacuole, and the enzyme linamarase is located in the cell wall [4]. Cassava roots are commonly processed before consumption to detoxify, preserve and modify it [5] into chips, *abacha*, *fufu*, tapioca, and *garri* [6].

Gari is granular starchy flour with slightly fermented flavour and a sour taste prepared from cassava mash [7]. Its particle sizes vary from one locality to another according to consumer preference [8]. It is the most popular form in which cassava roots are consumed in Nigeria [9, 10] due to its relatively long shelf life compared to other cassava food products along ide ease of preparation for eating [11]. *Gari* is mainly consumed as a meal or stiff dough (*eba*) prepared with hot water and taken with soup of all kinds or soaked in cool water with or without sugar and consumed with coconut, roasted groundnuts, dry fish, boiled cowpea or *moi-moi* as complement [10]. *Gari* has a high swelling capability and can absorb water up to four times its volume and contributes up to 60% of the total calories intake. *Gari* is mainly produced on a small scale and marketed by women as their source of income in many rural areas. In West Africa, there are two types of *gari* "white" and "yellow". Yellow *gari* is prepared by

toasting with some palm oil to give it a yellow colour while white *gari* is toasted without palm oil.

Characteristics taste and flavour of *gari* are developed during fermentation by lactic acid bacteria that produce volatiles like aldehydes, diacetyle esters and ethanol, as well as lactic acid [12]. Fermentation period correlates linearly with *gari* sourness and inversely with its pH [13]. Hydrogen cyanide (HCN) is liberated (reduced) during fermentation through spontaneous hydrolysis of the cyanogenic glycoside of cassava at low pH. *Gari* production reduces the hydrogen cyanide (HCN) content by about 50% [14]. Grating allows linamarase released from the cassava tissues to come in contact with cyanogen glycosides leading to rapid breakdown of sugar and cyanohydrins under neutral condition to hydrogen cyanide (HCN) and aldehyde or ketone [15, 16]. Hydrolysis of cyanogenic glucosides is cyanogenesis, [17] which is the most important contribution of fermentation. Traditionally, cassava mash for *gari* is given long fermentation to reduce hydrogen cyanide to a safe level [18].

Toasting pre-gelatinizes 60-80% of starch, decides the final quality of *gari* [19] reduces cyanide content further and finally reduces water activity of *gari*. Toasting *gari* with palm oil produces yellow *gari* (golden colour) which commands greater market value and appetite. Nutritionally, palm oil provides dietary energy, fat soluble vitamins, and serves as thermal insulators in the subcutaneous tissues [20]. Also, palm oil reduces (detoxify cyanide) the effect of hydrogen cyanide [21] and fights against vitamin A deficiency.

Cyanide is a chemical compound that contains the cyano group consisting of a carbon atom bonded by triple bonds to a hydrogen atom (C $\equiv$ N). The cyanide anion ($CN^{+)}$ is the primary toxic agent regardless of origin. When inhaled in the form of gas or ingested can activate some health complication in both human and animals [22, 23]. For an adult human, consumption of 50 to 100 mg or 2mmol of HCN within 24 hours can completely block cellular respiration leading to death [24]. Maximum recommended cyanide level of 20.0mg/kg for cassava products had been established [25].

Gari contains residual HCN which prolonged consumption or consumption of *gari* containing higher cyanide levels particularly in low protein diets have been reported to cause diseases such as tropical toxic neuropathy and goiter [22, 26]. Prolonged consumption of improperly processed *gari* can increase the cyanide contents in the human body and eventually cause goiter, cretinism, paralysis and neurological disorders [2]. Other symptoms of acute toxicity in human include dizziness, headache, nausea, stomach pains, diarrhea and sometimes death [27]. Cyanide acute toxicity may therefore be a problem in areas where cassava is the major source of calories. It is therefore imperative to reduce cyanide content of *gari* to a safe level before consumption; hence evaluation of cyanide levels in *gari* from five major producing areas in Abia State. This will help to identify the residual cyanide content levels of *gari*, enlighten the populace on the danger in prolonged consumption and how best to reduce it through processing like fermentation as a critical control point. Microbial load during handling, storage and exposure in the market will enlighten the populace on the dangers of unhygienic handling of *gari*. This study aimed at evaluating the residual cyanide, moisture content and microbial load of *gari* sold in different market locations in Abia State.

II. MATERIALS AND MEHTOD

A Sample Procurement

White and yellow *gari* used were bought from Ariaria , Isialagwa, Ndoro, Ndoki and Uzuakoli markets in Abia State. Three (3) samples were made from each market location at different times (March to June) and stored in air-tight containers for analyses.

B Sample Analyses

Determination of Cyanide Content

The residual cyanide levels of the *gari* were determined using the alkaline picrate method of Onwuka [28] with modifications. Five gram of each sample was dissolved in 50 ml distilled water and allowed to stay for 24h. The sample was filtered and the filtrate was used for the cyanide determination. To 1ml of the aqueous extract, 4ml of alkaline picrate (obtained by dissolving 1g of picrate and 5g of Na_2CO_3 in 200ml of distilled water) was added and incubated in water bath at temperature of 50^0 C for 5 minutes. The formation of a dark red colour was read spectrophotometrically at 490 nm against a reagent blank which contained 1ml of distilled water and 4ml of alkaline picrate solution. The experiment was repeated for three times and mean values were recorded for each sample.

Determination of Moisture Content

This was carried out according to AOAC [29] protocol. Five (5g) of each sample were placed into a previously washed, dried and weighed moisture can, dried in the oven at 105^0C for 3 and placed in a desiccator to cool. Thereafter it was returned to the oven for further drying, cooling and weighing repeatedly at an interval until a constant weight was obtained. The weight of the moisture lost was calculated as a percentage of weight of sample analyzed as expressed below.

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

Where W_1 = weight of the empty moisture can, W_2 = weight of moisture can + sample before drying and W_3 = Weight of moisture can + sample dried to constant weight.

Microbial Determination

Enumeration of microbial count in all the *gari* samples was carried out using surface spread plate method. Ten (10) grams of *gari* sample from each market locations were added to 90ml of 0.1% (w/v) sterile peptone water in a sterile 500ml beaker and allowed to stand with occasional stirring. Subsequently, 10 fold serial dilutions of sample were prepared and 0.1ml aliquots were spread on plate count agar for total heterotrophic bacteria count and same on potato dextrose agar for total fungal counts. Plates were incubated for 24 hours at 35^0C for bacteriological counts and for 3-5 days at 25^0C for fungal counts. Counts were expressed as colony forming units per gram of sample (cfu/g).

C Statistical Analysis

Data obtained from all the analyzed samples were subjected to analysis of variance (ANOVA) of a completely randomized design (CRD) using SPSS version 17.Treatment means were separated using Duncan Multiple Range Test (DMRT) at 95% confidence level ($p<0.05$).

III. Results and Discussion

Cyanide content

White gari

Results of the cyanide levels of white and yellow *gari* were presented in Table 1. Cyanide content of white *gari* varied with significant ($p<0.05$) difference between the market locations from 0.122 mgHCN/100g in Ndoki to 0.429mgHCN/100g in Isialangwa markets. Higher cyanide content of white *gari* from Isialangwa than the rest samples could be as a result of variation in fermentation period, cassava variety and soil chemistry of the areas involved. Toasting temperature and time [30] as well as stirring rate during toasting could also be a factor.

TABLE 1:
CYANIDE CONTENT LEVELS OF WHITE AND YELLOW *GARRI* SAMPLES OBTAINED FROM DIFFERENT MARKET LOCATIONS.

Sample source	Cyanide content levels (mgHCN/100g)	
	White	Yellow
Ariaria	$0.3255^{c} \pm 0.000$	$0.3045^{c} \pm 0.002$
Ndo	$0.3105^{b+} \pm 0.000$	$0.3105^{b} \pm 0.000$
Isialangwa	$0.4285^{a} \pm 0.003$	$0.3725^{a} \pm 0.003$
Uzoakoli	$0.1730^{d} \pm 0.003$	$0.1620^{d} \pm 0.0001$
Ndoki	$0.1220^{e} \pm 0.000$	$0.1210^{e} \pm 0.000$

Values are mean of three trails± standard deviation. Values with different subscript in same column are significantly different ($p<0.5$) and vice versa.

Yellow gari

Cyanide content of yellow *gari* sample from the five markets locations ranged from 0.12mg HCN/100g to 0.37mgHCN/100g with that from Isialangwa market location having the highest while that from Ndoki market location had the least. The disparity in cyanide content could be due to the level of oil added as well as aforementioned reasons in white *gari*. Reduction of cyanide content by palm oil had been acknowledged [21]. There were significant different ($p<0.05$) in cyanide contents of all yellow *gari* from different markets locations.

Generally, it is interesting to note that all the cyanide content of white *gari* were higher than their yellow counterparts except in Ndoro market location, but their difference were not significant ($p>0.05$). These results validated the report of Uvere [21] that palm oil reduces cyanide content. However, Results of moisture content of both white and yellow *gari* from the market locations are presented in Table 3.

White gari

Moisture content of white *gari* samples was highest (10.16%) in *gari* from Ndoro market location and least (6.32%) in *gari* from Ndoki market location. Moisture content levels of *gari* from all the market locations were lower than maximum 12% this notwithstanding, residual cyanide content of both white and yellow *gari* from all the market locations were lower than 20.0 mgHCN/100g recommended by FAO [11] and the lethal dose (50-60 mgHCN/1kg) for adults [31] who consumed the *gari* mostly. These implied safe for human consumption probably due to use of good cassava variety, adequate fermentation, toasting temperature and time as well as high stirring rate during toasting which must have reduced most of the cynaogenic glycoside in cassava [19, 18]. Cyanide is a toxic chemical compound which when inhaled in the form of gas or ingested can activate some health complication in both human and animals [22]. However, using improperly processed cassava can increase the residual cyanide contents in the human body and the associated ailment.

B *Moisture content levels*

recommended for shelf stable *gari* [32].There was significant ($p<0.05$) moisture content different between all the white *gari* samples sold in all the market locations which could be traced to differences in processing methods. Toasting temperature and time [30] as well as stirring rate employed could have a major effect on the moisture content.

TABLE 2:
MOISTURE CONTENT OF WHITE AND YELLOW *GARI* SAMPLES FROM THE MARKET LOCATIONS

Sample source	Moisture content (%)	
	White	Yellow
Ariaria market	$10.130^{a} \pm 0.028$	$11.255a \pm 0.021$
Ndoro market	$10.155^{b} \pm 0.021$	$10.155^{b} \pm 0.021$
Isialangwa market	$8.655^{c} \pm 0.021$	$7.325^{c} \pm 0.021$
Uzoakoli market	$7.910^{d} \pm 0.014$	$5.820^{e} \pm 0.014$
Ndoki market	$6.315^{e} \pm 0.007$	$6.880^{d} \pm 0.014$

Values are mean of triplicate determinations ± standard deviation. Values with same superscripts in same column are not significantly different. Values with different subscripts in same column are significantly different. Significant level is considered at 95% confident level.

Yellow gari

Location effects on moisture content of all the yellow *gari* samples varied from 5.82% in *gari* from Uzuakoli market location to 11.26% from Ariaria market location. There were significant ($p<0.05$) moisture different between all the yellow *gari* samples from all the market locations. The difference could be due to different toasting methods as explained in the moisture content of white *gari* and exposure to humid environment during handling (transportation, storage and market sales). Kaaya et al [33] had reported the inadequacy of storage structures commonly used by Nigerian farmers to maintain even, cool and dry internal atmosphere. This further indicates the tendency of the *gari* to grow mold. Higher moisture content encourages growth of microorganisms and therefore might not be favourable for prolong storage of *gari* [34, 35].

One important thing to note here is that moisture content does not depend on application of oil rather on locations which translates to difference processing methods and handling. Lower moisture content of both *gari* samples than maximum 12% recommended for shelf stable *gari* [32] indicated longer keeping quality provided they are stored in packaging materials that have low moisture permeability in a low relative humidity since *gari* is hygroscopic in nature. Besides, higher moisture content encourages microbial growth and lowers storage stability of *gari*, but when properly stored will keep up to 6 months or more [36].

B *Total bacterial and fungal counts*

Bacterial and fungal counts of white *gari* were presented in Table 3. Bacterial growth for the white *gari* sample from Ariaria market had the highest counts of 3.10 x 10^3cfu/g while that from Ndoro was the least (2.01 x 10^3cfu/g). The discrepancy could stem from variations in toasting, handling and humidity of the environment. Moisture content of the *gari* which among others depend on toasting temperature and time [30] as well as stirring rate may have contributed as moisture content of *gari* from Ariaria was higher than Ndoro market location (Table 3). In Ariaria market location, most of the *gari* sold there comes from mainly the traders from different places both far and near due to higher demand. Only few come from the producers which may have involved unwholesome handling. Again, Ariara market which is located on a table land without slope is always water logged which may likely increase the relative humidity. Conversely, *gari* from Ndoro market comes from only within the nearby villages mostly from the villagers that produced them which may result in proper handling. Ndoro market is water logged. There were significant ($p<0.05$) different between all the white *gari* samples from all the market locations which justified the influence of location on the quality of gari.

Fungal counts of the white *gari* samples from different market locations show that *gari* from Ariaria market location recorded the highest value with 2.70 x10^3cfu/g and lowest (1.16 0 x 10^3cfu/g) from Ndoki market location. The difference could be due to season of the year and differential residual moisture content of *gari* samples which among others may result from toasting temperature and time [30] as well as stirring rate during toasting. Higher moisture content (11.26%) of *gari* from Ariaria market location than 6.89% from Ndoki market location (Table 3) validated the difference. Fungi grow better in moist or humid environment as may be obtained in Ariaria market location due to its constant water logging during raining season. Differential relative humidity of the market locations and types of handling may as well contribute to differential fungi growth. There were significant ($p<0.05$) different between all the fungal counts of white *gari* sold in all the market locations. It is worthy to note that all bacterial counts were higher than fungal counts in all the white *gari* samples except in Ndoro market location which implies that bacteria counts depends on poor handling most especially during raining season.

TABLE 3:
TOTAL BACTERIAL AND FUNGAL COUNTS (X 10^3CFU/G) OF WHITE *GARI* SAMPLE FROM THE MARKET LOCATIONS.

Sample source	TBC(cfu/g)	TFC(cfu/g)
Ariaria market	$3.100^{a} \pm 0.000$	$2.700^{a+} \pm 0.000$
Ndoro market	$2.010^{b} \pm 0.000$	$2.175^{b} \pm 0.353$
Isialangwa market	$2.495^{c} \pm 0.007$	$1.680^{c} \pm 0.000$
Uzoakoli market	$2.200^{d} \pm 0.014$	$1.405^{d} \pm 0.106$
Ndoki market	$2.150^{e} \pm 0.000$	$1.160^{e} \pm 0.000$

Values are mean of triplicate microbial count ± standard deviation. Values with different subscript in same column are significantly different. Values with same subscript are not significantly different. Significant levels are considered at 95% confidence level ($p<0.5$).
TBC -Total Bacteria Count .TFC - Total Fungal Count.

Bacteria and fungi counts of yellow *gari* are presented in Table 4. Bacterial counts of the yellow *gari* samples from Ariaria market location had the highest counts of 3.20 x 10^3cfu/g while that from Ndoki market location had the least (2.400 x 10^3 cfu/g). Reasons as obtained in bacterial count in white *gari* and addition of oil during toasting may explain the difference. There were significant ($p<0.05$) difference in bacteria counts between all the yellow *gari* samples from different market locations which prefigured significant variations in toasting, oil addition and handling.

Fungal count of yellow *gari* samples from Ariaria market location was the highest ($2.90x10^3$cfu/g) while that from Ndoki market location was the least ($1.07x10^3$cfu/g). There were significant ($p<0.05$) difference in fungal counts between all the market locations. Reasons in fungal counts of white *gari* alongside addition of oil during toasting could explain the variations. Just like in white *gari*, bacterial counts of all yellow *gari* samples were higher than their counterpart fungal counts.

It is interesting to note that bacterial counts of all the yellow *gari* samples were higher than their white *gari* counterparts. This could be due to oil inclusion. Oil is an organic food component which is a good carbon source for microbial proliferation. Both fungal counts of white and yellow *gari* depended mostly on residual moisture content, season of the year and location. Higher moisture content and relative humidity (RH) of the location encourage fungal growth. The RH is higher during raining season. Despite all these, both bacteria and fungi counts were within the safe limit (10^4 to10^7 cfu/g) recommended by Center for Food Safety [37].

Bacteria are microscopic, single celled organisms that exist in their millions in every environment including inside and outside organisms. They feed on organic and inorganic compounds and can survive in extreme conditions. Harmful ones cause some diseases in humans like cholera, dysentery, pneumonia, typhoid and many more [38]. Fungi are free living microorganisms like molds, yeasts in soil or water in a parasitic or symbiotic relationship with plants or animals. Most parasitic infections that constitute major causes of death are found in less developed areas of the world [39].

TABLE 4
TOTAL BACTERIAL AND FUNGAL COUNTS (X10^3 CFU/G) OF YELLOW *GARI* SAMPLES FROM THE MARKET LOCATIONS.

Sample source	TBC(cfu/g)	TFC(cfu/g)
Ariaria market	$3.200^{a} \pm 0.000$	$2.900^{a+} \pm 0.565$
Ndoro market	$3.010^{b} \pm 0.000$	$2.175^{b} \pm 0.353$
Isialangwa market	$2.870^{c} \pm 0.000$	$1.800^{c} \pm 0.000$
Uzoakoli market	$2.450^{d} \pm 0.014$	$1.090^{d} \pm 0.127$
Ndoki market	$2.400^{e} \pm 0.000$	$1.070^{e} \pm 0.000$

Values are the mean of triplicate determinations ±standard deviations. Values with different subscript are significantly different ($p<0.5$) Values with same subscript are not significantly different. Significant levels are considered at 95% confidence level ($p<0.5$).TBC -Total Bacteria Count .TFC - Total Fungal Count.

This study revealed that all the cyanide levels of yellow *gari* from all the market locations were lower than their white counterparts due to addition of palm oil which indicated safer than the white *gari* samples. In contrast, microbial counts of the yellow *gari* samples were higher than their white counterparts. Moisture content levels of white and yellow *gari* samples varied with locations indicating variations in handling, toasting temperature and time as well as humidity of the areas.

Among all the market locations, white and yellow *gari* sample from Ndoki is the best with least cyanide value, total bacteria count, total fungal count and second to the lowest in moisture content. Following suit in this order is *gari* from Uzoakoli, Isialangwa, Ndoro,and Ariaria market locations. Despite the differences, both white and yellow *gari* from all the market locations understudied were safe for human consumption as they are below the recommended safe limit.

Low microbial count of *gar*i should be encouraged through proper handling by storing and selling in well packaged bags in areas devoid of humid environment. *Gari* should be toasted below safe moisture content limit of 12%. Lower cyanide content should be aimed at while preparing *gari* through adequate grating, fermentation and toasting with enough oil.

IV. CONCLUSION

This study revealed that all the cyanide levels of yellow *gari* from all the market locations were lower than their white counterparts due to addition of palm oil which indicated safer than the white *gari* samples. In contrast, microbial counts of the yellow *gari* samples were higher than their white counterparts. Moisture content levels of white and yellow *gari* samples varied with locations indicating variations in handling, toasting temperature and time as well as humidity of the areas.

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