Original Research Article

The predominant lactic acid microorganisms and proximate composition of spontaneously fermented *gari* and *fufu*, cassava food products

ABSTRACT

Aim: The present study is focused on determining if there are differences in the types of organisms responsible for spontaneous fermentation in two types of cassava food products, namely, *fufu* and *gari*, while also ensuring that the expected organoleptic properties associated with the fermentation process from this study location is reproducible.

Study Design: A Complete Randomized Design (CRD) with three replications was adopted and used to test for significant differences between the two cassava products.

Place and Duration of Study: The roots of two cassava varieties namely, TMS 97/0211 (white pulp) and TMS 97/2205 (yellow pulp) were obtained from the International Institute for Tropical Agriculture (IITA), Ibadan, and were processed at Ede, Nigeria between March and May 2016.

Methodology: Using standardized spontaneous fermentation methods, the two varieties of cassava, were sampled eight hourly over a period of 5 days, for lactic acid bacteria and fungi. Samples were incubated anaerobically, representative microbial populations were enumerated and identified using standard microbiological protocols. Proximate analysis and sensory evaluations were conducted.

Results: The results showed that the predominant lactic acid bacterial organisms were *Lactobacillus brevis* and *L plantarum*. On the other hand, the representative lactic acid fungal isolates were identified as *Neurospora crassa, Aspergillus fumigatus* and *Saccharomyces spp*. Investigation of succession organisms revealed differences between the dry cassava finished

product, *gari* and the wet finished product, *fufu.* The fungal organisms were the predominant starter organisms found in *gari*, while, the predominant starter organisms found in *fufu* were the bacterial types.

Conclusion: The present results show that in spite of the spontaneity of the fermentation process, the yellow cassava variety supports the growth and reproduction of similar fermentation organisms as the white variety. Furthermore, the prevailing microenvironment in the fermentation set up, that is, wet or dry is the most important factor in determining the predominating organisms in the fermentation process and the organoleptic and nutritional characteristics of the final product. Results from this study show that it is possible to reproduce the organoleptic and nutritional characteristics peculiar to this test location using the isolated lactic acid microorganisms.

Key Words: Food security, Lactic acid bacteria, *Lactobacillus brevis*, *Aspergillus fumigatus*

INTRODUCTION

Cassava (*Manihot esculenta*, *Crantz*) has been identified as a food security crop because of its potential to play a significant role in relieving the African food crises and as a constituent of foods for human nutrition and in the nutrition of livestock [1-5]. However, cassava and its food products are plagued by four important demerits, namely, a low energy density, low protein content, rapid postharvest deterioration and high cyanide content [3, 6]. Fermentation is used as a viable means of reducing the cyanide content, preserve and also to increase the nutrients in food products from cassava. In Africa, cassava fermentation is done by spontaneous fermentation and the array of organisms present in such fermentations is diverse based on variations in geographical locations and the quality of the substrates. As a result, the organoleptic and proximate characteristics of the final food product are oftentimes not reproducible. This is due to the spontaneous nature of the fermentation process. Many strains of organisms that produce the desirable biochemical changes such as enhancement of

the nutrient content of foods, essential amino acids and proteins, improvement of protein quality, fibre digestibility and degradation of anti-nutritional factors have been isolated and reported from cassava fermentation products [6 - 10].

Lactic acid bacteria (LAB) that have been reportedly isolated from cassava fermentation include members of the genera *Lactobacillus, Leuconostoc, Weissella* and *Bacillus* species [6, 11-16]. Other lactic acid organisms that have been reported from cassava fermentation includes, yeasts from genera such as *Saccharomyces, Pichia, Candida* and *Trichosporon* species [15 - 19].

Fufu and *Gari* are food products from cassava with differences in the fermentation processes whereby they are made into finished products. *Gari* is processed by squeezing water out of peeled cassava root (de-watering) and the resulting cassava pulp is left to ferment for several days and then sieved and fried to dryness to make the final product. On the other hand, *fufu* is processed by peeling the cassava tubers and cutting them into pieces of 2-5cm^3 and left to ferment. These are then submerged in water as the product ferments until preparation with boiling water to make the pasty final product [20-23]. These differences in processing are expected to affect the diversity and abundance of the fermentation organisms and consequently on the physicochemical, microbiota and organoleptic properties of the finished product.

The fermentation method; wet or dry as found in *fufu* and *gari* respectively have been reported to affect the type and predominance of isolated fermentation organisms and this in turn, affects the organoleptic appeal of the finished product [22-23]. The present study compares lactic acid fermentation in dry (*gari*) and wet (*fufu*) processed cassava food products. This is expected to give insight into whether or not the cassava processing method may significantly affect the type of fermenting organisms found on the product and the effect of these on organoleptic appeal and the proximate parameters. The organoleptic appeal of the

final product was also compared with the type and number of the isolated fermentation organisms. This is with the aim of reproducing organoleptic and nutritional characteristics peculiar to this test location by utilizing the identified organisms as starter organisms in the fermentation process.

MATERIALS AND METHODS

Collection of Samples

Two varieties of cassava TMS 97/0211 (yellow inner root color) and TMS 97/2205 (white inner root color) were obtained from the International Institute of Tropical Agriculture Ibadan, Oyo State, Nigeria. Tubers from these two varieties of cassava were processed based on the flow chart shown in Chart 1 (a and b) for *fufu* and *gari* respectively. Samples for microbial and proximate analyses were taken during the fermentation stages for both food products. The fermentation stage lasted for 5 days in both cases and samples were taken 8-hourly from the commencement of fermentation.

Identification of Isolates

Microbiological analyses were conducted immediately after sampling by suspending 10 g of the root pulp in 90 ml of sterile normal saline, vortexed and further diluted in a 10-fold dilution series and 0.1 ml of suitable dilutions. For Lactic acid bacteria, inocula were spread onto De Man Rogosa Sharpe (MRS) agar, plates were incubated anaerobically at 30°C for 24 h in an anaerobic incubator (Surgical Medical England Model SM-80CH, uv). Representative dominant colonies were picked from the plates of the suitable dilutions and purified by repeated streaking onto nutrient agar. For lactic acid fungi, inocula were spread onto potato dextrose agar (PDA). Eight hourly changes over a period of 5 days in the microbial population of the total viable lactic acid bacteria and fungi were determined using MRS agar

and PDA respectively. Samples were enumerated by using appropriate sterile dilution and spread plate methods eight hourly. For the identification of microbial isolates, the fungal plates were incubated at 25°C for 2-5 days, while the bacteria were incubated at 30°C for 24-48 h. Three colonies for each morphological type were purified and maintained in the appropriate agar plates. Systematic morphological and biochemical tests were conducted according to Cowan and Steel [24], moreover, identification of bacterial isolates into species was done according to tests and descriptions as earlier provided [25, 26]. The fungal isolates were characterized by their cultural properties stained with cottonblue lactophenol solution and observed microscopically [27, 28].

Proximate and organoleptic analysis

The proximate composition of each sample of gari was determined using standard analytical procedures. The amount of HCN was calculated in milligram per kilogram of *gari* or *fufu* based on Association of Official Analytical Chemists (AOAC) method [29]. The ash content of the samples was also determined similarly using methods described in AOAC. After burning the food sample on a Bunsen burner and incinerating the charred material in a muffle furnace set at 550ºC until a whitish grey ash remained, then the residue was cooled in a desiccator and weighed [29]. The percentage moisture content of the food sample was determined based on weight loss of water due to evaporation during drying in an oven at 50ºC for four hours until constant weight was obtained. The soxhlet extraction method as described [30] was used in determining the crude fat. The extraction under reflux was carried out with petroleum ether at a temperature range of 40-60ºC for 5 hours, followed by drying in an oven for 30 min at 100ºC for the solvent to evaporate, cooling and weighing. Crude fibre was determined as difference between the oven dry weight and weight after ashing divided by the sample weight while crude protein was determined using the Kjedahl method [29].

For the sensory evaluation (colour, aroma and texture), the *gari* or *fufu* obtained on zero fermentation was poured into container labelled 0th day, *gari/fufu* of the 1st day of fermentation into container labelled 1st day, gari of the 2nd day of fermentation into the container labelled 2nd day, and so on till all the six containers were filled with *gari/ fufu*. A panel of thirty individuals were invited for the sensory evaluation (organoleptic appeal) of odour, taste, appearance, pasting, texture and general acceptability. The samples in the container were presented to the evaluators at random. The evaluators were asked to award scores for each sample after observing the colour, aroma and texture of each sample. The products were ranked on a scale of 1-5; 1 – extremely dislike, 2- dislike, 3- neither like nor dislike, 4- like and 5- like extremely.

Experimental Design

Complete Randomized Design (CRD) with three replications was used to test if spontaneous fermentation of cassava improves the proximate characteristics of cassava food products such as the study on the effect of duration of fermentation on moisture content, ash content, crude fibre, crude protein, and reduces the anti-nutrients including poisonous substances such as hydrogen cyanide (HCN). The results of the three replicates were pooled and expressed as mean ± standard error (S. E.). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $p \leq 0.05$ using SPSS software version 21.0.

RESULTS

Isolation and identification of lactic acid bacteria and fungi from the fermented products

Table 1 shows the identification table of representative lactic acid bacterial isolates from the anaerobic culturing of samples from the fermentation of cassava for *gari* and *fufu* for the two varieties of cassava, namely, TMS 97/0211 characterized by yellow inner root color and TMS 97/2205 characterized by white inner root color. The representative isolates

were grouped based on cultural characteristics, gram staining and biochemical test results. The results showed that irrespective of the variety of cassava or the fermentation method, the predominant organisms remained the same. The organisms were identified as *Lactobacillus brevis* and *L plantarum*.

The identification table of representative lactic acid fungal isolates from the anaerobic culturing of samples from the fermentation of cassava for *gari* and *fufu* for the two varieties of cassava (Table 2). The representative isolates were grouped based on cultural, morphological characteristics and results of standard biochemical reaction. The results showed that irrespective of the variety of cassava or the fermentation method, the predominant organisms remained the same. The three (3) organisms were presumptively identified as *Neurospora crassa, Aspergillus fumigatus* and *Saccharomyces spp*.

Succession of organisms

Table 3 shows the percentage frequency of isolation of the organisms encountered during the spontaneous fermentation process for gari. The fungal organisms identified as *Aspergillus fumigatus, Neurospora crassa* and *Saccharomyces spp* were the predominant starter organisms isolated from the *gari* samples with incidence values ranging from 0.1 – 0.6 x 10^{-8}cfu/ml of samples as shown in Fig. 3 (a and b). These ranges were consistently obtained for samples obtained from both the yellow and white varieties. However, the amount of occurring lactic acid bacteria identified as *Lactobacillus brevis* and *L plantarum* increased as the fermentation progressed between days 2 and 3 in particular. These bacterial organisms appeared to finish off the fermentation earlier initiated by the fungal isolates which predominated earlier during the fermentation process. Conversely, the lactic acid bacterium *Lactobacillus brevis*, was more predominant in the fermentation of *fufu*, followed by the fungus, *Aspergillus fumigatus*, although just like in the case of *gari* fermentation, the

bacterial organisms increased in the concluding part of the fermentation as the number of fungi gradually reduced as shown in Figs 2 (a and b).

As shown in Table 4, fermentation of the cassava for five days caused a significant increase ($P \leq 0.05$) in the protein ash and fat content in all the processing pathways to which the samples were subjected, whether fufu or gari and regardless of the type of variety. The results of the cyanide content of both the *fufu* and *gari* samples before and after fermentation are also presented in Table 4. Fermentation of the cassava caused a significant decrease ($P \leq 0.05$) in the cyanide content of the two varieties of cassava for both the *fufu* and *gari* treatments.

Table 5 shows the results of the organoleptic tests on *fufu* and *gari* samples processed from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color). The results showed that for odour, the yellow *gari* made into a popular form in which *gari* is eaten, known as *eba* (*gari* stirred into boiling water to make a firm dough which may be eaten with the desired soup) was the more preferred for general acceptability. On the other hand, the white *fufu* was the more preferred for general acceptability. The indicated values are average scores of triplicates, n=30. However, there were no significant differences when the recorded values were compared statistically at $p \leq 0.05$ using a one-way analysis of variance ANOVA.

DISCUSSION

The identities of the fermentation organisms isolated from the present study confirm the results from similar studies that were done elsewhere on cassava fermentation [6, 18, 20, 31, 34]. Beyond the foregoing, the present result goes further to show that even though there may be differences in taste due to the type of processing method employed since there is a fundamental difference between the taste of *fufu* and *gari*, the fermenting organisms were the same. The final differences in taste could however be attributable to differences in metabolic

products in the *gari* processing pathway which is dryer and the *fufu* processing pathway which is fundamentally wet and water-submerged from start to finish. Submerged fermentation as obtained in the processing of *fufu* is generally known to produce mash which contains a foul odour and is known to cause more variations in the final product [32, 33]. The present results show that the fundamental difference in the taste of these cassava food products may not be as a direct result of the activities of the fermentation organisms.

The results from the present study demonstrate the proof of principle that fermentation of cassava helps to reduce the cyanide content, preserve and also increase the nutrients in food products from cassava (Tables 4 and 5). This shows that the isolated lactic acid organisms are capable of utilizing cyanogenic glycosides and the breakdown products during cassava fermentation and have good potential for development into commercial products that can be used for commercial *fufu* and *gari* processing operations. Even though the cyanide levels in the unfermented varieties of cassava used in the present study are well below the detrimental level of 30mg/kg [9- 10, 16], fermentation by the lactic acid organisms further reduced the cyanide levels to make the final products safer for human consumption.

The succession data presented in this study (Figs 2 and 3; Table 3) shows that the lactic acid bacteria, *Lactobacillus brevis*, L. *plantarum* and lactic acid fungi, *Aspergillus fumigatus*, *Neurospora crassa* and *Saccharomyces spp* are promising candidates for subsequent pilot studies in order to optimize the organoleptic and nutritional characteristics of *gari* and *fufu*. This is well corroborated by previous reports where *L. plantarum*, selected as a starter culture for the production of sour cassava starch in pilot-scale fermentation process exhibited better and faster acid production among the isolated bacteria and was recommended as the starter culture [16, 18, 34]. In fact it was concluded that the success of *L. plantarum* to predominate in cassava fermentation demonstrates the potential for its development as a starter culture for *gari* industrialization. Moreover, the success of the use of lyophilized LAB

222 strains as starter cultures for *gar*i production and the discovery that *L. plantarum* could be
223 produced at low cost has been reported [34]. While *L. plantarum* is one of the two lactic acid
224 bacteria isolated from the present study, *L. brevis* seemed to be more predominant. This is not
225 surprising however, since the microbiota from spontaneous cassava fermentation is known to
226 be from various origins and may come from raw materials, utensils and equipment used in its
227 production. Insects or handlers can also carry these microorganisms and may well account for
228 the observed variation [18].

229 The present result showing similarity in the type and amount of isolated lactic acid
230 microorganisms from both the yellow and white cassava varieties is valuable to the
231 development of the yellow cassava variety as a high quality variety of cassava globally.
232 These results further confirm that the acidic environment created by lactic acid bacteria not
233 only favours the proliferation of lactic acid fungi but also provides growth factors such as
234 vitamins and compounds that have significant impact on organoleptic and nutritional quality
235 of food [8]. Although the complex interactions between lactic acid bacteria and fungi are not
236 yet fully understood, it is well known that lactic acid bacteria and fungi have the ability to
237 adapt to many different substrates [35]. Traditionally, yellow *gari* is made by adding red
238 palm oil to white *gari* during the frying stage of processing. This genetically modified variety
239 however makes it possible to obtain yellow *gari* without the oil adding step. The organoleptic
240 data from the present study confirms that food products from the yellow variety of cassava
241 compares very well to the better known white variety of cassava in terms of proximate
242 analyses (availability of nutrients in the fermented final product) and results of overall
243 organoleptic appeal to consumers (Tables 4 and 5).
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CONCLUSION

The present results show that in spite of the spontaneity of the fermentation process, there are similarities in the type and amount of isolated lactic acid microorganisms from both the yellow and white cassava varieties. This result confirms that lactic acid bacteria and fungi have the ability to adapt to many different substrates. In addition, the results show that the prevailing microenvironment in the fermentation set up, that is, wet or dry is the most important factor in determining the predominating organisms in the fermentation process and the organoleptic and nutritional characteristics of the final product. Moreover, the present results demonstrate the successful isolation of the lactic acid bacteria, *Lactobacillus brevis*, L. *plantarum* and lactic acid fungi, *Aspergillus fumigatus*, *Neurospora crassa* and *Saccharomyces spp* as promising candidates for subsequent pilot studies in order to optimize the organoleptic and nutritional characteristics of *gari* and *fufu*. These results indicate that it is possible to reproduce the organoleptic and nutritional characteristics peculiar to this test location with the aid of the identified lactic acid microorganisms.

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Authors' contribution

This work was carried out in collaboration between the authors. Author A (**Dr. Femi Ayoade**) designed the study, performed laboratory and statistical analyses, wrote the protocol, and wrote the first draft of the manuscript. Author B (**Paulina Adeniji**) procured the cassava varieties and performed the proximate analysis in the laboratory, Author C (**Kellany Amole**); Author D (**Yeitarere Amaremo**); Author E (**Titilayo Apata**); Author F (**Scott Fayemi**); Author G (**Nicholas Oyejide**); Author H (**Uchenna Abazu**); Author I (**Tolulope Kayode**); Author J (**Gbenga Daramola**) performed sample collection and laboratory analyses of the study. Author K (**Onikepe Folarin**) contributed to the development of protocol and provided technical support. All authors read and approved the final manuscript.

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Competing Interests

The authors declare that they have no competing interests

Figure 1: Flow Chart for the processing of cassava in to *gari* and *fufu*

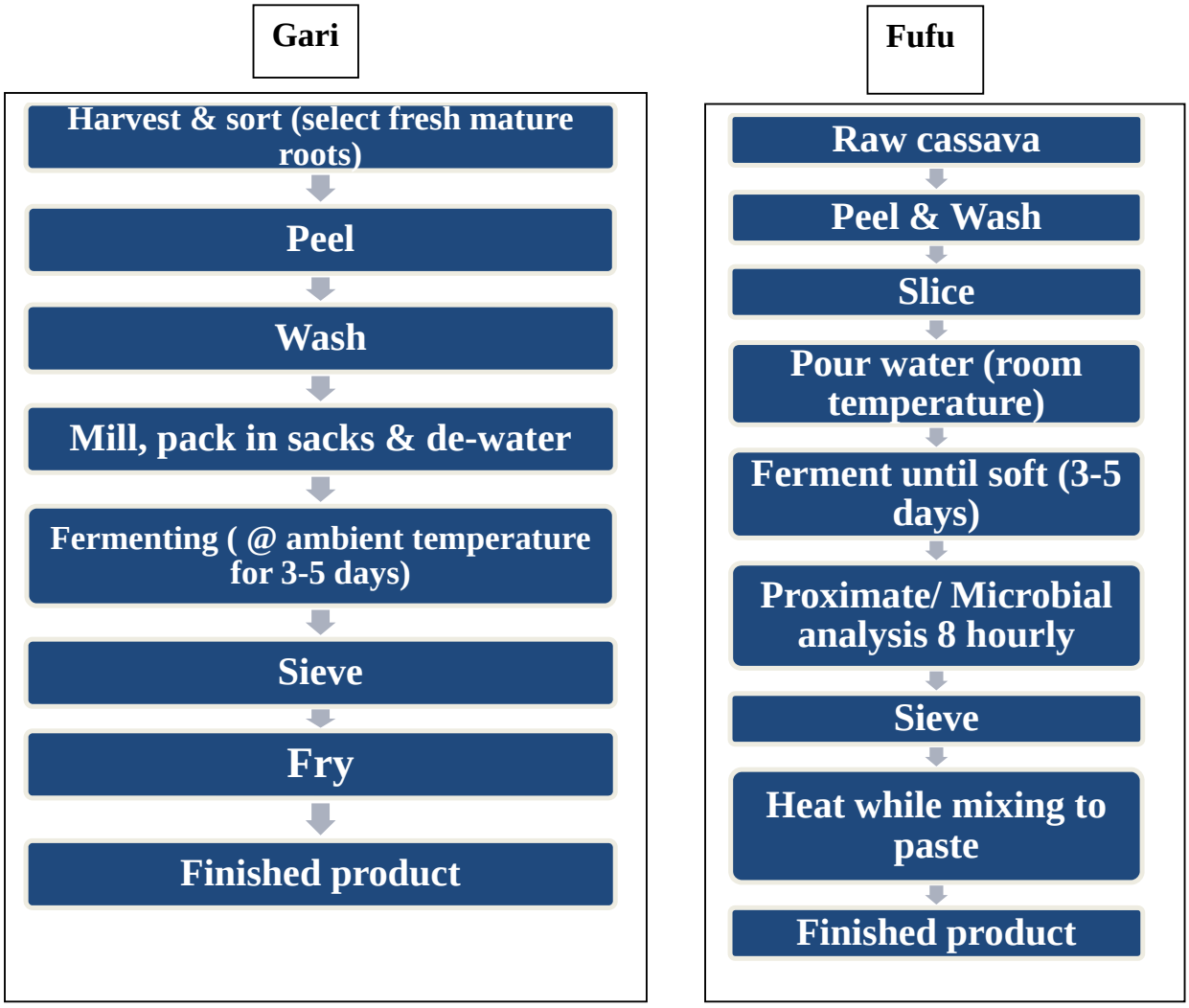

Fig 2a: A line graph tracking the typical incidence/ abundance ($x10^{-8}$ *cfu/g)* of the lactic acid bacteria from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color) during *fufu* fermentation.

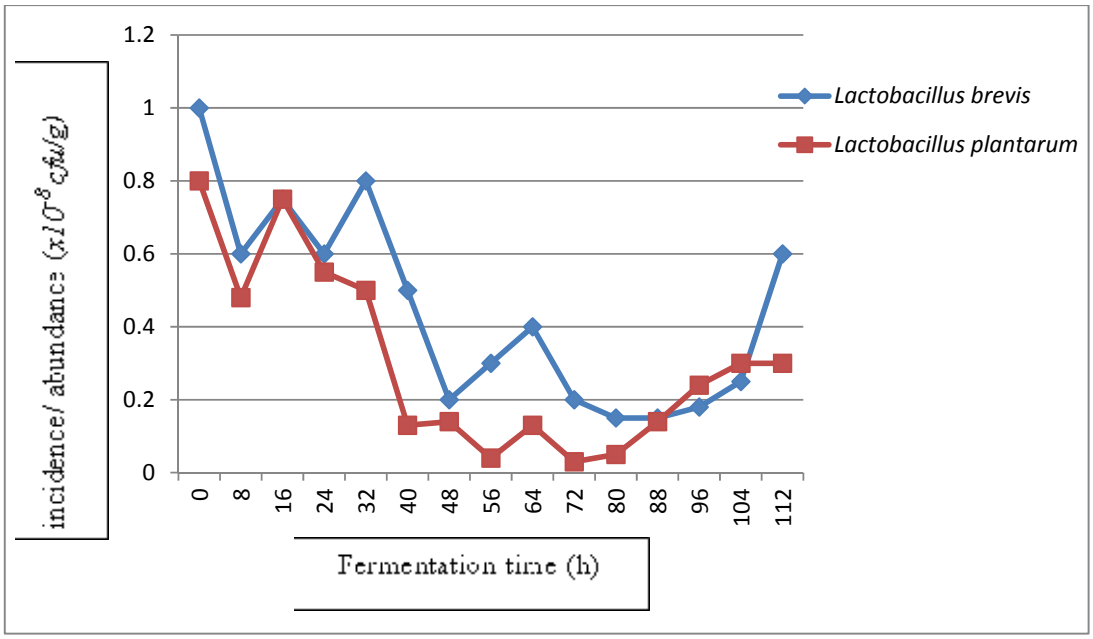

Fig 2b: A line graph tracking the typical incidence/ abundance ($x10^{-8}$ *cfu/g)* of the lactic acid fungi from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color) during *fufu* fermentation.

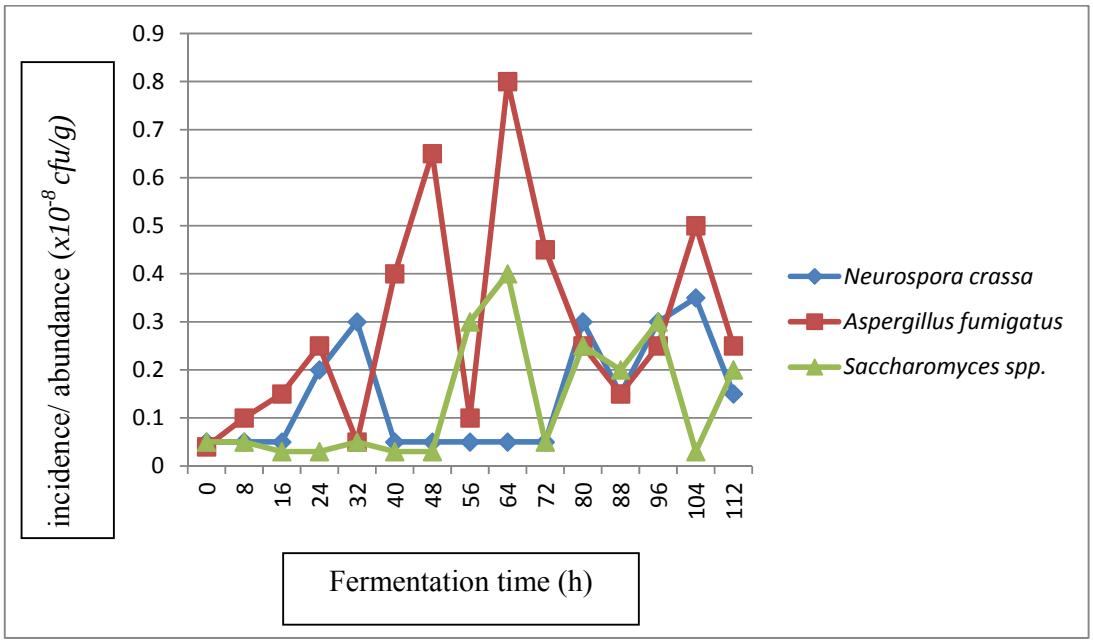

Fig 3a: A line graph tracking the typical incidence/ abundance (*x10^{-8} cfu/g)* of the lactic acid bacteria from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color) during *gari* fermentation.

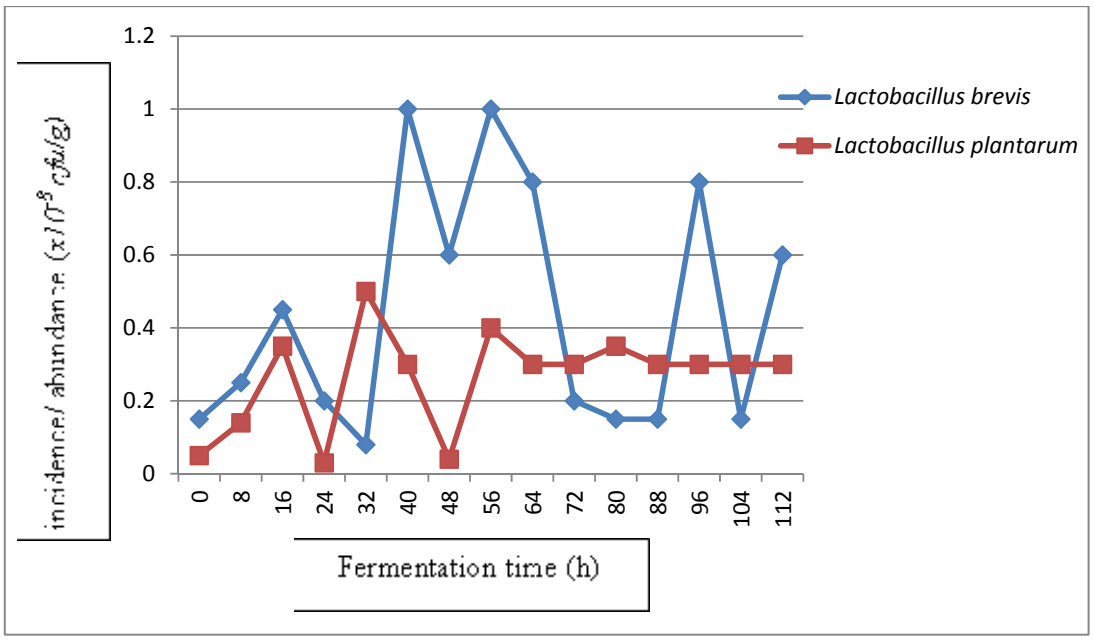

Fig 3b: A line graph tracking the typical incidence/ abundance (*x10^{-8} cfu/g)* of the lactic acid fungi from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color) during *gari* fermentation.

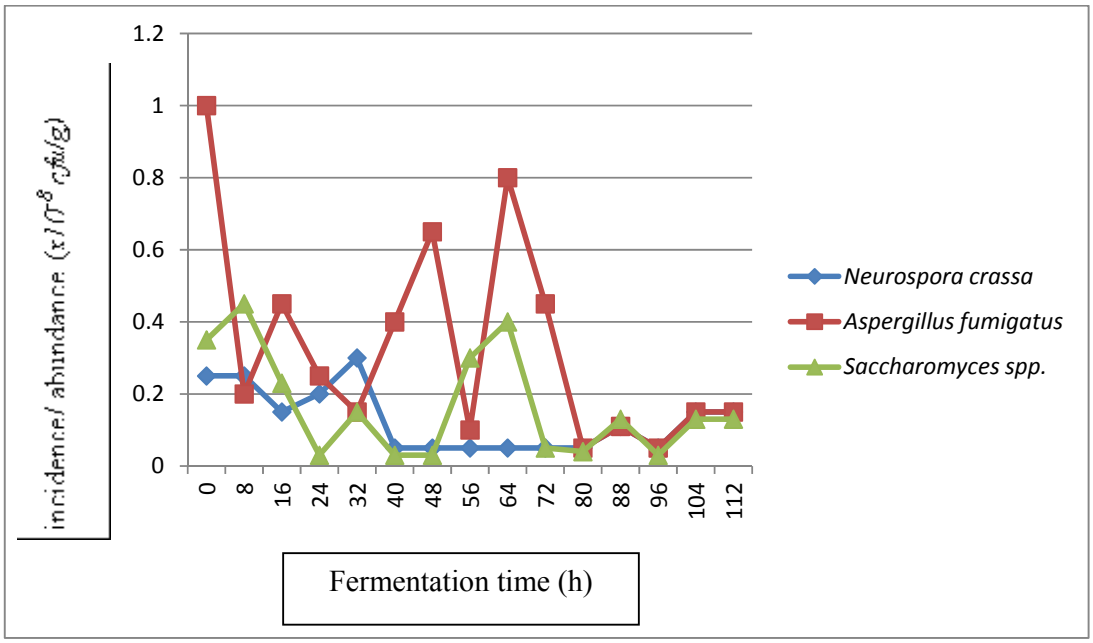

Table 1: Identification table of bacterial isolate- gari/ fufu processed from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color)

Gram staining	Morphology	Catalase	Methyl Red	Arginine Decarboxylase	Glucose	Fructose	Sucrose	Lactose	Maltose	Ribose	Arabinose	Mannitol	Suspected organism
+	Rod	-	+	+	+ No gas	+ No gas	+ No gas	+ No gas	+ No gas	+ No gas	+ gas	+ No gas	*Lactobacillus plantarum*
+	Rod	-	+	+	+ gas	+ No gas	+ gas	+ No gas	+ No gas	+ No gas	+ No gas	+ No gas	*Lactobacillus brevis*

Table 2: Identification table for the fungal isolates from both varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color)

Organism	Morphological Characteristics	Microscopic Morphological Characteristics	Identified Organism
1	**White wooly mycelia, same on reverse plate.**	**Septate hyphae, distinctively longitudinally ribbed with nerve-like ridges in sac-like organ (Ascus)**	***Neurospora crassa***
2	**Black spores with cream mycelia edges, same on reverse plate.**	**Hyphae is septate. Spore bearing.**	***Aspergillus fumigatus***
3	**Cream yeast-like spores, same on reverse plate.**	**Hyphae is non-septate. Conidiophores are branched and smooth. Head is radiated.**	***Saccharomyces spp***

Table 3: Typical succession data expressed in percentage (%) frequency of isolation of organisms observed during the spontaneous fermentation of yellow and white cassava varieties processed as *gari* or *fufu*

0-12 hrs post fermentation		12-36 hrs post-fermentation		36 – 48 hrs post – fermentation		36 – 48 hrs post – fermentation	
Bacterial organisms	Fungal organisms	Bacterial Organisms	Fungal organisms	Bacterial organisms	Fungal Organisms	Bacterial organisms	Fungal organisms
Lactobacillus brevis (5)	*Aspergillus fumigatus* (45)	*Lactobacillus brevis (40)*	*Aspergillus fumigatus (5)*	*Lactobacillus brevis (48)*	*Aspergillus fumigatus* (5)	*Lactobacillus brevis (50)*	*Aspergillus fumigatus (10)*
Lactobacillus plantarum (5)	*Neurospora crassa (25)*	*Lactobacillus plantarum (40)*	*Neurospora crassa (10)*	*Lactobacillus plantarum (30)*	*Neurospora crassa (5)*	*Lactobacillus plantarum (30)*	*Neurospora crassa (5)*
	Saccharomyces spp (20)		*Saccharomyces spp (5)*		*Saccharomyces spp (12)*		*Saccharomyces spp (5)*

Table 4: Proximate composition of *fufu* and *gari* before and after the fermentation processes (expressed in ppm) processed from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color)

Sample	Protein (%)	Crude fibre (%)	Ash (%)	Fat (%)	Carbohydrate (%)	HCN*
White gari	6.2 ± 0.4^{b} (10.5 ± 0.2^{e})	3.9 ± 0.5^{d} (2.3 ± 0.3^{a})	1.3 ± 0.2^{a} (2.6 ± 0.3^{b})	2.6 ± 0.5^{a} (6.7 ± 0.5^{d})	87.5 ± 1.2^{b} (76.6 ± 3.2^{a})	**6.7986 (2.3980) (35.3%)**
Yellow gari	7.1 ± 0.3^{b} (11.2 ± 0.4^{e})	2.7 ± 0.3^{b} (1.9 ± 0.1^{a})	0.9 ± 0.3^{a} (2.7 ± 0.4^{b})	1.3 ± 0.2^{a} (4.1 ± 0.4^{b})	91.4 ± 0.6^{c} (79.2 ± 1.2^{b})	**6.2980 (2.6810) (42.6%)**
White Fufu	4.6 ± 0.4^{a} (9.1 ± 0.2^{c})	2.9 ± 0.4^{c} (1.3 ± 0.2^{a})	1.2 ± 0.3^{a} (2.9 ± 0.4^{b})	1.3 ± 0.3^{a} (4.6 ± 0.4^{c})	90.5 ± 1.2^{c} (75.8 ± 2.1^{a})	**6.5980 (2.8850) (43.7%)**
Yellow Fufu	5.1 ± 0.3^{b} (10.4 ± 0.3^{d})	2.7 ± 0.3^{b} (1.9 ± 0.2^{a})	1.1 ± 0.4^{a} (3.1 ± 0.2^{b})	1.2 ± 0.2^{a} (5.1 ± 0.3^{c})	91.6 ± 1.2^{c} (74.6 ± 1.3^{a})	**7.7430 (2.5250) (32.6%)**

*Each value represents the mean of 3 replicates; the final values are in parentheses. The percent change in HCN levels (mg HCN equivalents/ 100g) are expressed also in parentheses. The results of the three replicates were pooled and expressed as mean ± standard error (S. E.). A one-way analysis of variance (ANOVA) and the least significance difference (LSD) were carried out. Significance was accepted at $p \leq 0.05$.

Table 5: Organopleptic appeal test results of *fufu* and *gari* processed from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color)

Sample	Odour	Taste	Appearance	Pasting	Texture	General Acceptability
White gari (*Eba*)	3.7	4.0	4.1	4.2	4.3	**4.2**
Yellow gari (*Eba*)	3.8	3.9	3.8	4.1	4.1	**4.3**
White *Fufu*	3.8	4.1	4.3	4.5	4.4	**4.5**
Yellow *Fufu*	4.1	4.3	4.1	4.4	4.1	**4.1**

The indicated values are average scores of triplicates, n=30. However, there were no significant differences when the recorded values were compared statistically at $p \leq 0.05$ using a one-way analysis of variance ANOVA.