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Original Research Article

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

ABSTRACT

Cassava as a food security crop, is plagued by four important demerits, namely, a low energy density, low protein content, rapid postharvest deterioration and high cyanide content. The present study investigates spontaneous lactic acid fermentation in two types of processed cassava food products, namely, *fufu* and *gari*. Using standardized spontaneous fermentation methods, root pulps from two varieties of cassava, namely, TMS 97/0211 and TMS 97/2205 were sampled eight hourly over a period of 5 days, for lactic acid bacteria and fungi. Samples were incubated anaerobically, representative dominant colonies were picked from the plates of the suitable dilutions and purified by repeated streaking onto nutrient agar. Microbial populations were enumerated and identified using standard microbiology methods. Proximate analysis and sensory evaluations were conducted. 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*. Succession studies 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*, conversely, the predominant starter organisms found in *fufu* were the bacterial types. Results from the present study confirmed the proof of principle that fermentation enriches food value, organoleptic appeal and lowers anti-nutrients and toxigenic food chemicals such as hydrogen cyanide. The

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present study is of unique value to the development of yellow cassava variety as a high quality variety of cassava globally.

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

INTRODUCTION

Cassava (*Manihot esculenta*, *Crantz*) has been described as a 'food security crop' because of its ability to grow extensively throughout the tropic and sub-tropic regions in diverse soil conditions and with minimal management [1, 2]. Moreover, apart from cassava being a cheap source of carbohydrate, it is the main carbohydrate source in the diet of the teeming population of the third world countries where it is mostly grown [3]. Food security is a condition that exists when individuals at all times have economic and physical access to safe, nutritious and sufficient food in order to meet their dietary needs and food preferences for an active and healthy life [4]. Cassava has been known to play a significant role in relieving the African food crises as a constituent of foods for human nutrition and in the nutrition of livestock. In Nigeria in particular, cassava is the primary food source that feeds about 200-300 million animals and people. Nearly all Nigerian farmers plant cassava along with other crops because it resists disease, drought and pest outbreaks more than other crops [5].

In spite of the importance of cassava as a food security crop, it is plagued by four important demerits, namely, a low energy density, low protein content, rapid postharvest deterioration and high cyanide content [3, 6]. Fermentation is traditionally used as a viable means of reducing the cyanide content, preserve and to increase the nutrients in food products from cassava. Traditionally, cassava fermentation is done by spontaneous fermentation under varying hygienic conditions. Usually, the array of organisms present in such fermentations is diverse based on variations in geographical locations and the quality of the substrates. Some

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of these organisms are undesirable, producing anti-nutrient and other metabolites contrary to the purpose of fermentation. Some of these undesirable organisms are sometimes pathogenic or responsible for food deterioration and spoilage. The strains of organisms that produce the desirable biochemical changes are those that release amino acids, ammonia and other volatile compounds. Many of these have been isolated and reported from cassava fermentation products [6, 7] however, in Africa, spontaneous fermentation is typically used in these fermentation processes and organisms with potentials for use as starter cultures in locally fermented foods abound and should be well studied. During fermentation, the nutrient content of foods is enhanced through the biosynthesis of vitamins, essential amino acids and proteins, thereby improving protein quality and fibre digestibility. Fermentation also enhances micronutrient bioavailability and aids in degrading anti-nutritional factors [8, 9].

Lactic acid bacteria (LAB), that have been reportedly isolated from cassava fermentation includes members of the genera *Lactobacillus, Leuconostoc, Weissella* and *Bacillus* species [6, 10]. Other lactic acid organisms that have been reported from cassava fermentation includes, yeasts from genera such as *Saccharomyces, Pichia, Candida* and *Trichosporon* species [11, 12, 13].

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 [14, 15]. These differences in processing is expected to affect the diversity and abundance of the fermentation organisms

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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 [16]. 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. The isolated organisms were identified and it is expected that these organisms may be further developed as industrial fermentation products utilizable in cassava processing.

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 at Oyo road, 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.

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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 was purified and maintained in the appropriate agar plates. Systematic morphological and biochemical tests were conducted according to [17], moreover, identification of bacterial isolates into species was done according to tests and descriptions given in [18] and [19]. The fungal isolates were characterized by their cultural properties stained with cottonblue lactophenol solution and observed microscopically [20, 21].

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 [22]. 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

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muffle furnace set at 550ºC until a whitish grey ash remained, then the residue was cooled in a desiccator and weighed [22]. 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 by [23] 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 30m ins 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.

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, rude protein, and reduces the anti-nutrients including poisonous

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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 strain 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, namely, TMS 97/0211 and TMS 97/2205 is shown in 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

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Aspergillus fumigates, 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. These ranges were consistently obtained for samples obtained from both the yellow white varieties. However, the amount of occurring lactic acid bacteria identified as *Lactobacillus brevis* and *L plantarum* increased as the fermentation progressed. These bacterial organisms appeared to finish off the fermentation earlier initiated by the fungal isolates. 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 were predominant in the concluding part of the fermentation as the number of fungi gradually reduced.

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

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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 similar studies that were done on cassava fermentation, notably, the works of [6, 14, 24] identified similar organisms from spontaneous fermentation of cassava. 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 [25, 26]. 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 to 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], fermentation by the lactic acid organisms further reduced the cyanide levels to make the final products safer for human consumption.

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The present result is of unique value to the development of the yellow cassava variety as a high quality variety of cassava globally. Traditionally, yellow *gari* is made by adding red palm oil to white *gari* during the frying stage of processing. This genetically modified variety however makes it possible to obtain yellow gari without the oil adding step. The organoleptic data from the present study confirms that food products from the yellow variety of cassava compares very well to the better known white variety of cassava in terms of proximate analyses (availability of nutrients in the fermented final product) and results of overall organoleptic appeal to consumers (Tables 4 and 5).

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

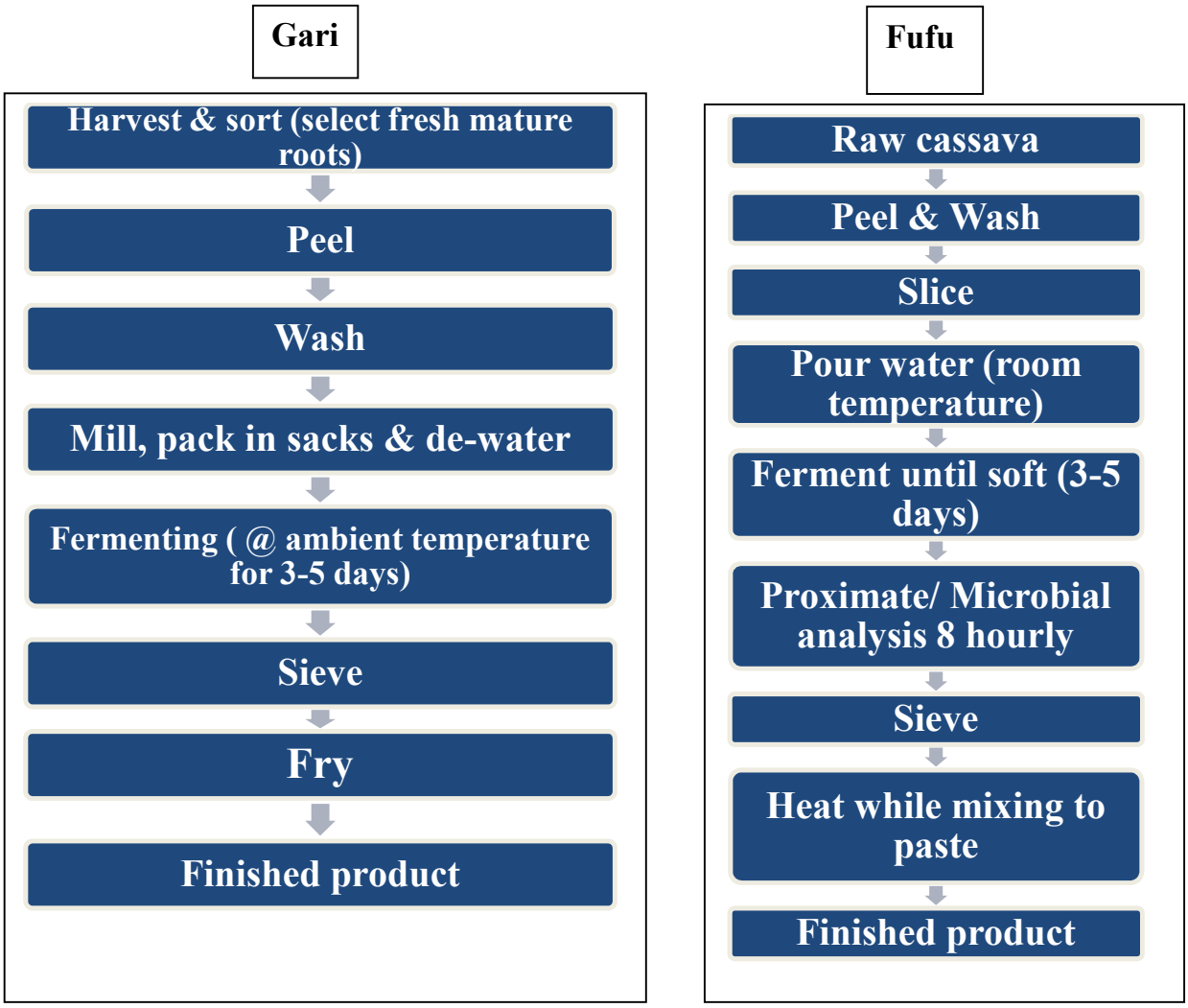

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Table 1: Identification table of bacterial isolate- gari/ fufu processed from two varieties of cassava (TMS 97/0211; yellow inner root color and TMS 97/2205; white inner root color)

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***

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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 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

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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.