

Determination of Starter Cultures (Organisms) in fermentation of Cassava (*Manihot esculenta*), Used for Fufu Production.

ABSTRACT

Fufu is a traditional Nigerian fermented cassava food product. Due to the production of objectionable odor, fermentation was done in the Laboratory using improved techniques. Sweet white, Yellow, bitter cassava varieties were used. Since a wet fufu mash is accomplished by various microorganisms, this work looked into the contents of the cassava mash for several days to determine the starter microorganisms. The microbial, chemical and sensory changes during the fermentation were determined. Microbial counts were higher as retting progressed, but reduced at completion. Heterotrophic bacterial counts decreased from 2.65-2.46Log₁₀CFU/mL, for sweet variety, but increased from 1.95-2.27 Log₁₀CFU/mL for bitter variety; 2.28-2.59 Log₁₀CFU/mL for yellow variety on Nutrient agar. Coliform counts decreased from 2.56 to 2.28Log₁₀CFU/mL for sweet variety, but increased from 2.32 - 2.55Log₁₀ CFU/mL for bitter variety, from 2.30 - 2.49Log₁₀ CFU/ mL, for yellow variety on MacConkey agar and from 1.91 - 2.41Log₁₀CFU/mL, for sweet variety, from day 2; 1.91-2.23 Log₁₀CFU/ML, for bitter variety, from day 3), and 2.10 – 2.32 Log₁₀CFU/mL (for yellow variety, from day 3) for Fungi on SDA. The dominant Starter organisms were a mixed population of heterotrophic bacteria, *Bacillus* spp, yeasts and mould. Protein content of the sweet variety increased from 0.3- 5.25% and the yellow variety from 0.2- 4.375%, their cyanide contents reduced from 2.7- 0.01mg/kg and from 4.3 - 0.04 mg/kg respectively, showing lost of hydrogen cyanide. Cyanide content of bitter variety remained 10.6mg/kg at the end of fermentation, since there was neither retting nor lost of hydrogen cyanide. The pH of the product reduced as the fermentation progressed. Sensory evaluation of the fermented samples accepted all the samples, but liked most, the sweet white variety. The bitter variety was not assessed because it did not ferment even on day 5. These organisms can be used as starter cultures to improve the protein content of fufu, and reduce the cyanide content to minimal level which makes it safer for consumption.

Keywords: Starter organisms, Hydrogen cyanide, *Bacillus* spp., Cassava, Fufu

1.0. INTRODUCTION

Cassava (*Manihot esculenta crantz*) which is known locally as “Jiakpu”, is a perennial shrub with an edible starchy root, which grows in the tropical and subtropical areas of the World [1]. Cassava is one of the most important food crops for about 200-300 million people and some animals. It provides a basic diet for over 800 million people worldwide. It is a key to national food security in Nigeria. Cassava known as, “green gold” is regarded as a better option to “black gold” (petroleum), and contributes greatly to gross domestic product (GDP) of the nation [2]. In 1999, Nigeria produced 33 million tons of cassava and being the world’s highest producer of cassava [3, 4] Imo State is the third largest producer in Nigeria and it sustains the livelihood of indigenous communities. It is the basis of many products including food in Africa and Latin America. Cassava is mostly used as food for human consumption, while in Asia and other parts of Latin America it is used commercially for the production of animal feeds and other starch-based industrial products [5].

Cassava is a major staple food in developing countries. It is estimated that the crop provides about 40% of all the calories consumed in Africa and ranks second only to cereal grains as the chief source of energy in Nigerian diet [6], by this, cassava plays an important role in alleviating Africa food crises. It is rich in cyanide (>10mg/100g fresh weight) and poor in protein (1.2%) for some varieties [7]. It has been variously used in the production of different types of food in Africa such as garri, fufu (akpu), lafun, abacha and tapioca [8].

Cassava is normally processed before consumption as a means of detoxification, preservation and modification [9]. It undergoes detoxification to reduce the toxic effect in the roots due to the presence of cyanogenic glycosides, especially linamarin. Fermentation which is the most important processing method for the crop can be classified into solid state (without soaking in water) as in garri and submerged fermentation (soaking in water) as in fufu production [10].

Fufu is a fermented cassava food product which comes as a wet mash or dry powder [11]. It ranks next to garri as an indigenous cassava food for most Nigerians in the South-South and Eastern zones. Fufu is a staple food of many countries in Africa and the Caribbean. It is often made with flour or paste made from the cassava plant or alternatively flour such as: maize flour or semolina. Fufu stands out especially in Ghana and West Africa generally. The name fufu was derived from the Twi word “Fufuo” of the Akan in Ghana meaning “white”, due to its appearance. Fufuo is actually the correct way to refer to the dish. Among the Baule and other Akan groups in Cote d’Ivoire, it is known as Sakora, among the Dagombas of Northern Ghana, it is known as Sakoro, and as couscous (couscous de Cameroun) in the French speaking regions of Cameroon. The main ingredient is usually cassava. In Ghana, before cassava was introduced to Africa from Brazil by Portuguese traders in the 16th century, fufu was made with yam. In some situations, it is made with plantain or cocoyam. In Nigeria, Togo, and Cameroon, fufu is white in color and sticky (if plantain is not mixed with the cassava when pounding) [12].

Caribbean fufu (*fufu de platano*) is different from West African fufu both in the texture and the flavorings. Caribbean fufu and motongo (Puerto Rico) are less of a dough-like and more of a firm consistency, while the African fufu is more of a dough-like [13].

Fufu is a very good source of carbohydrate (about 98%) and energy, but very low in protein (about 2.0%), therefore, there is a great need to improve the protein content of fufu because of the need to produce food rich in protein to feed the increasing population of the world. Since the bulk of diets of many people in the population consist mainly of carbohydrate foods that are low in protein, protein deficiency diseases like kwashiorkor, coronary diseases, etc abound [14].

During fermentation of cassava tubers for fufu production, there exists a succession of organisms in the fermenting mash, some of which may not be needful in the system; hence, Lactic acid bacteria, yeast, and other bacteria contribute significantly to starch breakdown, acidification, detoxification and flavour development [9]. Another potential problem in processed fufu is the flavour of the product, which may be undesirable to many people. The fermentation process is initiated as a result of chance inoculation by microorganisms from the environment. The presence of unspecified microorganisms complicates the control of the fermentation process and leads to the production of objectionable odour. Such problems have led to the development of several other processing techniques using starter culture suitable for odorless fufu [15, 16]. Okolie *et al.*, [17] proposed that the microbiological process should be modification in order to upgrade the cassava product, but much attention has not been paid to it.

Based on these, this research was embarked on with the aim to determine starter organisms (cultures) for the production of odorless fufu, enriching the protein content, reducing cyanide level and developing starter organisms(cultures) for fermenting cassava tubers for wet fufu mash production. The starter organisms (cultures) are microbiological cultures that initiate and assist fermentation [18]. They are formed using a specific culture medium and a specific mixture of fungal and bacterial strains, for instance: *Aspergillus spp.*, *Mucor*, *Amylomyces spp.*, *Endomycopsis spp.*, *Saccharomyce spp.*, *Lactobacillus spp.*, *Acetobacter spp.*(Boulton and Quain; Mullan; YAmamoto and Fujiuchi).

Three (FAO, 2007) main types of starter organism used commercially in Australia, UK, North America, Nigeria and New Zealand are (a) Single-strain starters (b) Multi-strain Starters (c) Mixed-strain starters. Single-strain starters involve a single strain of micro-organism like *Lactobacillus coryeformis*, *Saccharomyces cerevisiae*, *Candida utilis*, etc, in the production of a particular fermented food, example *Streptococcus lactis* (*Streptococcus cremoris*) used singly or in pairs in the production of cheeses, etc. Multi-strain starters are starters with defined mixtures of three or more single strains of a particular species of micro-organisms. Multi-strain (multiple strains) starters are frequently referred to as mixed strain starters in the United States of America [19]. Thermophilic cultures such as *Streptococcus thermophilus* and *Lactobacillus delbrueckii ssp bulgaricus* are used in the production of yoghurt, acidophilic milk, swiss like cheese. They thrive together with milk and become a good starter culture for yoghurt production (32).

2.0 MATERIALS AND METHODS:

Collection of cassava tuber samples

Approximately, 20kg of the roots of one year old cassava tubers of the species: Provitamin (Vitamin A, Mucas 26), TMS198/0505 (White sweet variety), and NR 8082 (White bitter variety) were collected from the farm at the premises of National Root Crops Research Institute, Umudike, Abia State, Nigeria, identified by the Agricultural Development Project, Owerri, Imo State, Nigeria, as the yellow roots, and onuawuru for the white species. Samples were immediately transported to the Microbiology Laboratory of the Department of Medical Laboratory Science, Imo State University, Owerri, for processing.

Processing of the cassava tubers

The method of wet fufu production described by (11) was modified to ret the tubers to obtain wet fufu mash in the laboratory. The tubers were harvested, sorted, peeled with knife, cut into cylindrical portions (4-7cm long) and washed with sterile tap water. Three kg of the peeled tubers of each of the three varieties were soaked separately in 5 liters of sterile water using three different plastic buckets with lid for 4 days. The retting ability and the pH of the samples were assessed daily. The organisms in the retting water were isolated daily, characterized and identified. At the end of the four days, the retted tubers were washed, mashed in clean water and sieved through a normal fine mash to remove the fibers from the vascular bundles. The filtrate was allowed to settle and excess water decanted. The three different wet fufu mashes were separately transferred into clean

Jute bags and the remaining water pressed out [9]. All the fufu samples produced were tested for crude protein content and total cyanide content. Also, sensory evaluation of the fufu mash was done and the data analyzed statistically.

Analysis

Various analytical methods were employed to analyze some parameters in the wet fufu samples. The parameters analyzed in this research work using standard laboratory procedures included: The retting ability of the tubers, pH of the fufu samples, crude protein content, total cyanide content, bacteria and fungi contents and organoleptic qualities of the wet fufu samples.

Total Microbial counts, Identification of the bacterial and fungal isolates

Bacterial and fungal isolates were identified each day based on the method of the International Commission on Microbiological Specification of Foods [20]. A unit value of the samples (1mL) was aseptically transferred into a clean sterile petri dish. 19 mL of the molten Nutrient agar, MacConkey agar and Sabouraud Dextrose agar was aseptically poured into the petri dish containing the three different samples separately. They were mixed clockwise, anti-clockwise, front and back for 3 times each, to enhance even distribution of the samples. They were allowed to solidify. The inoculated plates were incubated at 37°C and room temperature respectively. The number of colonies in each of the triplicate plates of each sample was counted with the aid of a colony counter. The bacterial load was calculated as the total viable count expressing the number of colony forming unit/mL of the sample as: **Equation:** TVC (CFU/mL) = $\frac{1}{V} \times N$

TVC = Total viable count, V = Volume of inoculums, N = no. of colonies

Determination of the retting ability of the tubers

Procedure: With the help of a sterile disposable hand glove, the retting ability of the tubers was manually determined with the covered hand by feeling the degree of softness of the tubers.

pH estimation of the fufu

The pH of the fufu was determined using Suntex pH meter. The probe of the pH meter was calibrated using buffer solution (pH 7.0) for alkalinity and (4.0) for acidity. The probe was rinsed with distilled water and wiped to remove excess water at the end of calibration. 10grams of each of the three fermenting cassava mash (wet fufu mash) were separately collected and homogenized differently in 100mL of sterile distilled water in a beaker. The probe of the pH meter was inserted into the sample being measured. The pH values of the samples were read and recorded [21].

Crude protein content estimation

The Method of Kjeldahl described by Bradley, [22] was adopted to determine the protein content of the wet fufu mash samples. The nitrogen content was determined and multiplied by the factor 6.25 to obtain protein content in the samples. One gram of the processed samples was digested by boiling in 10mL of concentrated H₂SO₄ in the presence of Selenium catalyst in a fume cupboard until a clear solution was obtained. The digest was diluted into 100mL volume flask. A portion, 10ml of the digest was mixed with equal volume of 45% NaOH solution and digested in a semi micro Kjeldahl apparatus, the distilled sample was collected into 10mls of 4% boric acid solution containing 3 drops of mixed indicator (methyl orange and bromocressol green). A total of 50mls of distilled sample was collected and

titrated against 0.02N H₂SO₄, from green to a deep red colour change was observed. A reagent blank was also digested and treated the same way. The formula below was used to calculate the protein content.

Equation: % protein = $V_o N_2 \times 6.25$

$$V_o N_2 = 100 \times \frac{14 \times N \times V_f \times T - B}{W \times 1000 \times V_a}$$

Key: W = weight of analyzed, N = Normality of titrant solution, V_f = Total volume of digest, V_a = Volume of digest distilled, T = Titre of sample, B = Titre of reagent blank.

Cyanide content estimation

The total cyanide content was determined using the alkaline picrate colometric method as described by Balagopalan *et al.*, [23] 1g of the processed sample was dispersed in 150mls of distilled water in a 250ml conical flask. A strip of wet alkaline pikrate paper was suspended inside the flask without touching the water surface with help of rubber bung or cork on the flask. A standard cyanide solution (1ml) was prepared and diluted to a recurred concentration; it was treated the same way in a separate flask. The flasks were incubated over night at room temperature (18-25°C). The next day, the pikrate papers were carefully removed and eluted in 60mls of distilled water in a separate flask. Their respective absorbance was measured in a Spectrophotometer at 540nm wavelength with the reagent blanked at zero. The cyanide content was determined by the formula below:

Equation: % Cyanide content mg/dl = $1000 \times \frac{a_u}{W} \times \frac{C}{a_s} \times D$

Key: W = Weight of sample analyzed, a_u = absorbance of test sample, a_s = absorbance of standard cyanide solution, C = Concentration of the standard mg/dL, D = Dilution factor where applicable

Determination of the organoleptic qualities

The 9-point hedonic scale described by EL-Tinay *et al.*, [24] was used. This was done to determine the colour, texture, aroma, taste and general acceptability of the three processed fufu samples. Fifteen students who are conversant with the odour, texture and other qualities of fufu were randomly selected from Imo State University, Owerri to perform the sensory evaluation.

Each panelist was seated separately to avoid influences on their scoring from other judge. Each of the panelist was given a sample of the product, a glass of water to rinse his or her mouth after each tasting to avoid interference with the taste, and was asked to commit on how much he/she liked or disliked the product by rating the sample on the bases of the given parameters using 9-point hedonic scales shown below:

9-Like extremely. 8-Like moderately. 7-Like very much. 6-Like slightly. 5-Neither like nor dislike, 4-Disliked slightly, 3-Disliked very moderately. 2-Disliked very much, 1-Dislike extremely.

The panelist evaluated the samples for taste, colour, texture and general acceptability and the result of the test was assessed using the HEDONIC Scale.

3. RESULTS

3.1 Microbial counts

The data from this study are presented as bar charts in figures 1a-c, 2 and tables 1a, b, 5. Figures 1a-c represent the Log_{10} of Total bacterial, Coliform and Fungal counts for the 4 days of retting of the different cassava varieties.

Figure 1a represents total bacterial counts of the varieties per day of retting. Total bacterial counts decreased from Log_{10} 2.65- 2.46CFU/mL for sweet variety, increased from Log_{10} 1.95- 2.27CFU/mL for bitter variety and Log_{10} 2.28-2.59 for yellow variety on nutrient agar. Total coliform counts decreased from Log_{10} 2.56 - 2.28CFU/mL for sweet variety, increased from Log_{10} 2.32-2.55 CFU/mL for bitter variety and log_{10} 2.30-2.49 CFU/mL for yellow variety on day 2, whereas bitter and yellow varieties increased from Log_{10} 1.91-2.23 and Log_{10} 2.21- 2.32 on MacConkey agar respectively on day 3.

Morphological and Biochemical criteria for the identification of bacterial and fungal isolates are shown in tables 1a-b, while the organisms and their prevalence is presented in figure 2.

The results of retting ability are presented in table 2, whereas pH changes, crude protein/cyanide contents and Organoleptic qualities are shown in figures 3-5.

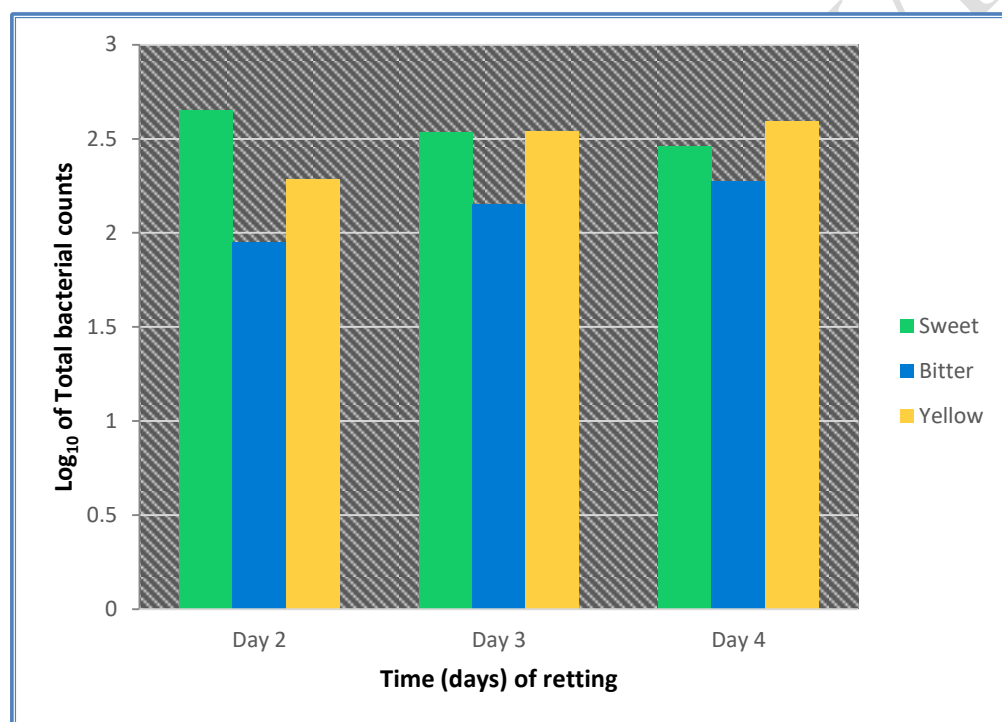


Figure 1a: Log_{10} of the total bacterial counts per days of retting

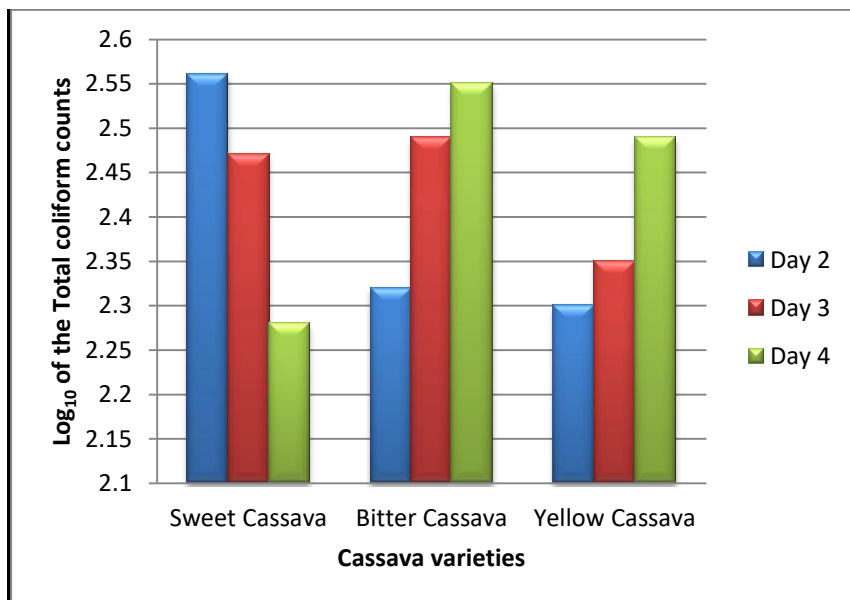


Figure 1b: Log₁₀ of the Total Coliform counts from the cassava varieties per day.

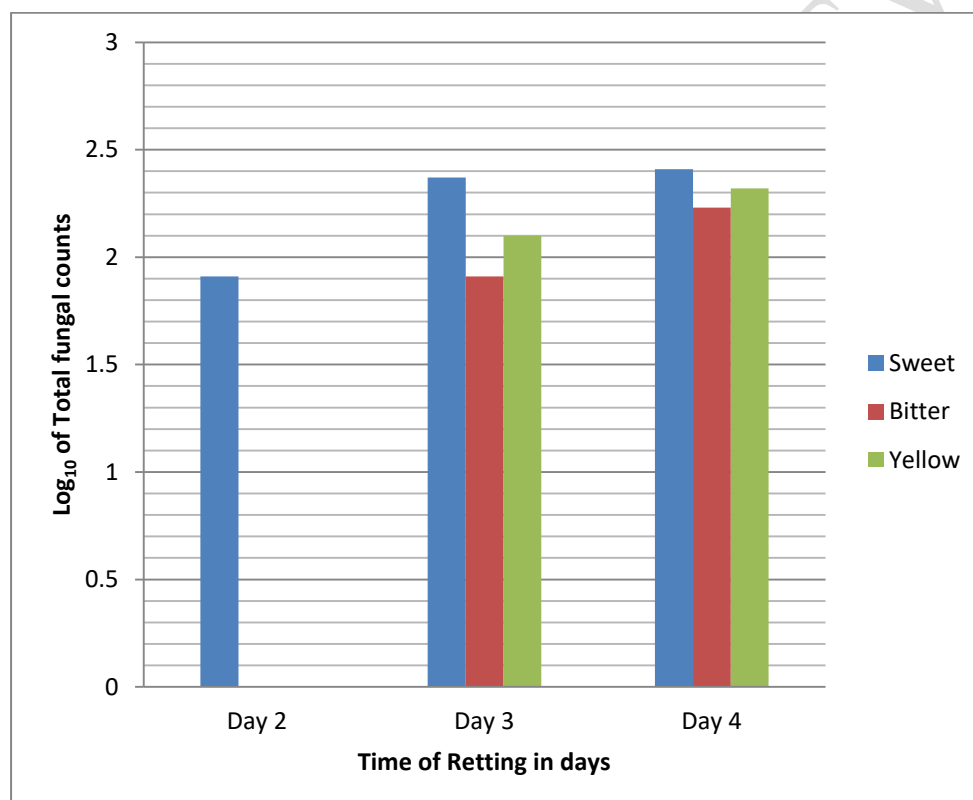


Figure 1c: Total Fungal counts from the cassava varieties per day

Table 1a: Bacterial isolates from the three samples and Identification criteria

Gen Region	Motility	Sol fermentation	Gelatin	Catalase	Indole test	Citrate	Urease	H ₂ S	MR	VP	KN	Glucose	Lactose	Maltose	Sucrose	Mint	Gelatin	Oxidase	Optim
-short rods	-	-	-	-	+	-	-	-	-	-	-	A	A	-	A	A	-	-	<i>Escherichia coli</i>
-rod in industrial and single	-	-	+	+	-	+	-	-	-	-	-	A	-	-	-	-	-	+	<i>Syngnathus</i>
-long rods in chains	+	+	+	-	-	+	-	-	+	-	-	AG	-	-	-	-	-	-	<i>Bacillus subtilis</i>
-short thick rods	-	-	+	-	-	+	+	-	-	+	+	G	A	-	A	-	-	-	<i>Klebsiella aerogenes</i>
-rods	+	-	+	-	-	+	-	-	-	-	+	AG	-	-	-	-	-	+	<i>Bacillus anthracis</i>

Key: -Negative, + = Positive, G= Gas production, AG= Acid and Gas Production.

Table 1b: Fungal isolates and Identification criteria

Cult ural Char acter istics	Cell Mor phol ogy	Assimilation of Sugar							Fermentation of Sugar							Org anis m
		Gl uc os e	M alt os e	La ct os e	Ga lac tos e	M ai nt ol	Su cr os e	De xtr os e	Gl uc os e	M alt os e	La ct os e	Ga lac tos e	M ai nt ol	Su cr os e	de xtr os e	
Smoo th, crea my white , hairy colon ies	Bud ding cells	+	+	-	-	-	+	-	+	+	-	-	+	+	-	<i>Saccharo myces cerevisiae</i>
White, crea m flat smoo th colon ies	Oval budd ing cells with pseu dohy phae	+	+	-	-	-	+	-	+	+	-	+	-	+	+	<i>Candida albicans</i>

Colony that appeared whitish when young (48-72hrs), and brownish black, double branching, septate hyphae under the microscope after 72-96 hours. A powdery texture, soft and smooth growth after 72-96 hours of incubation at room temperature suggested *Aspergillus spp.*

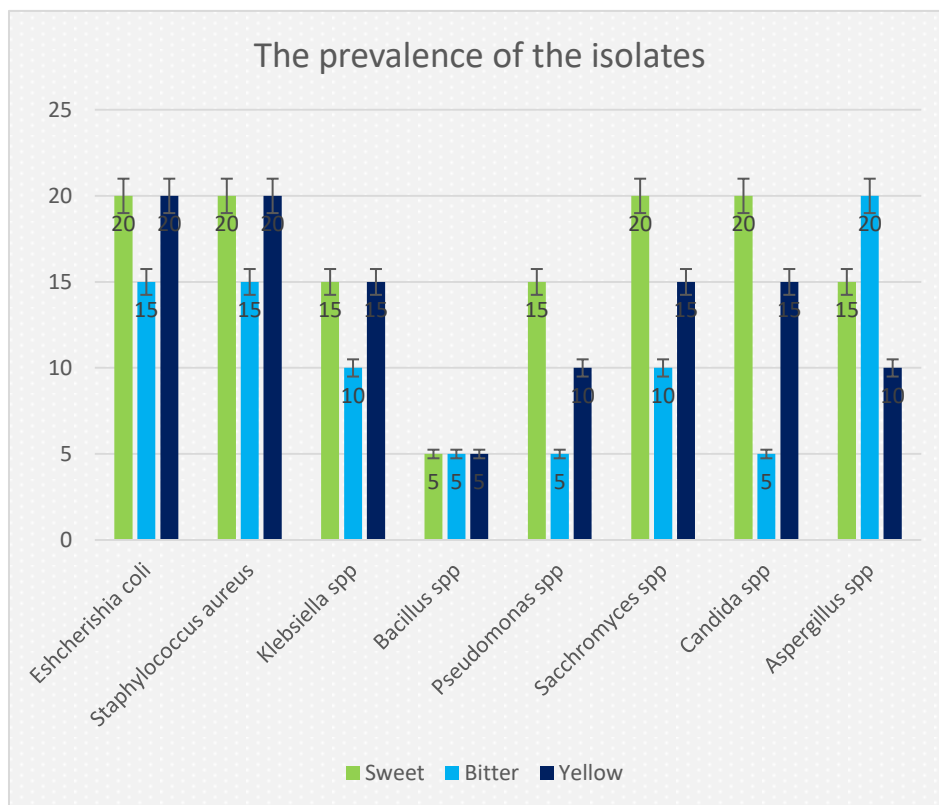


Figure 2: Organisms identified from the products of the three cassava varieties and their prevalence (%).

Y-axis = Prevalence (%) of the Organisms, X- axis=Organisms

Calculation equation: Prevalence of Isolates =

$$\frac{\text{No. of plates that is positive for a particular organism}}{\text{No. of sample tested}} \times 100$$

All the organisms were isolated from the retting water of all the varieties, but at varying prevalence. The prevalence of the organisms ranged from 5-20%, with *Escherichia coli*, *Staphylococcus aureus*, *Sacchromyces ssp*, and *Candida spp*; *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus spp*. had the highest prevalence(20%) in sweet ; yellow and Bitter varieties respectively, while *Bacillus spp* recorded the lowest(5%) in all of them.

Table 2: The retting ability of the tubers

Days	Yellow Variety	Sweet Variety	Bitter Variety
Day 1	-	±	-
Day 2	-	++	-
Day 3	+	+++	-
Day 4	++	+++	-
Day 5	+++	+++	-

Key: - = No retting, + = Partial retting, ++ = complete retting, +++ = fully fermented

For Sweet Cassava variety, retting started from the first day and was fully fermented on day 3, whereas for Yellow variety, retting started on day 3 and full fermentation occurred on day 5. Because the bitter white variety was not fermented till the fourth day, they were allowed to

stay for more days in water to ascertain the day on which fermentation would occur before washing and sieving in water.

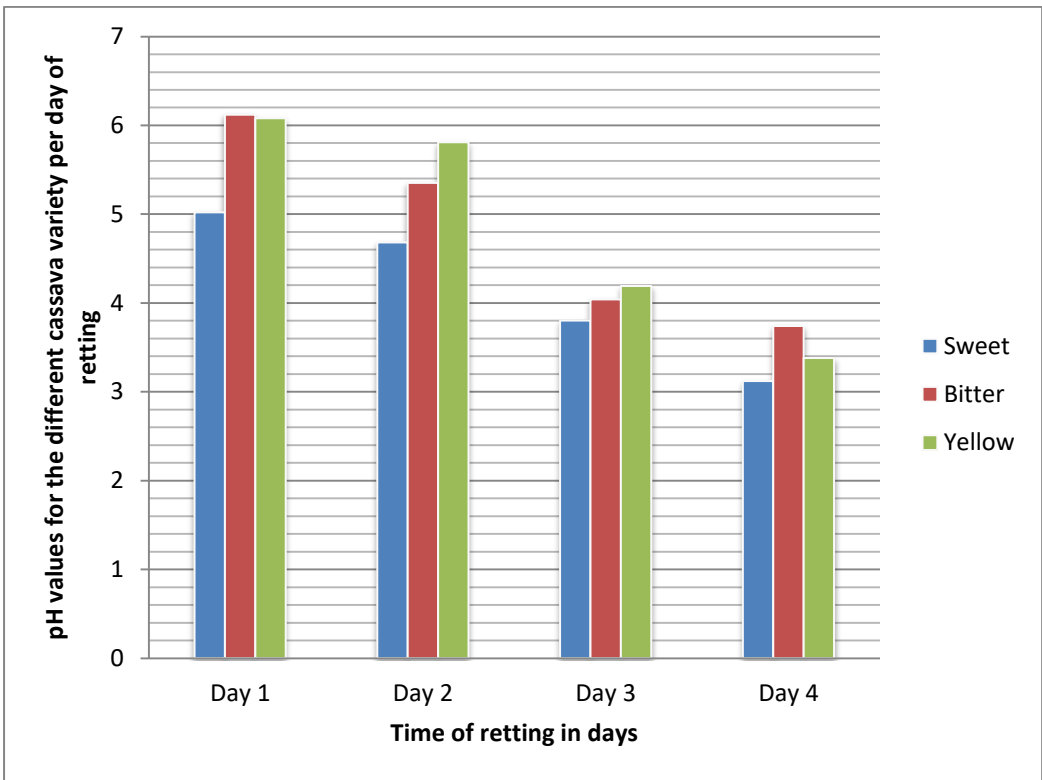


Figure 3: Changes in the pH values of the retting water from the three cassava varieties. pH values decreased with fermentation date, i.e. Retting water became more acidic as fermentation progressed; highest for bitter variety and lowest for sweet variety.

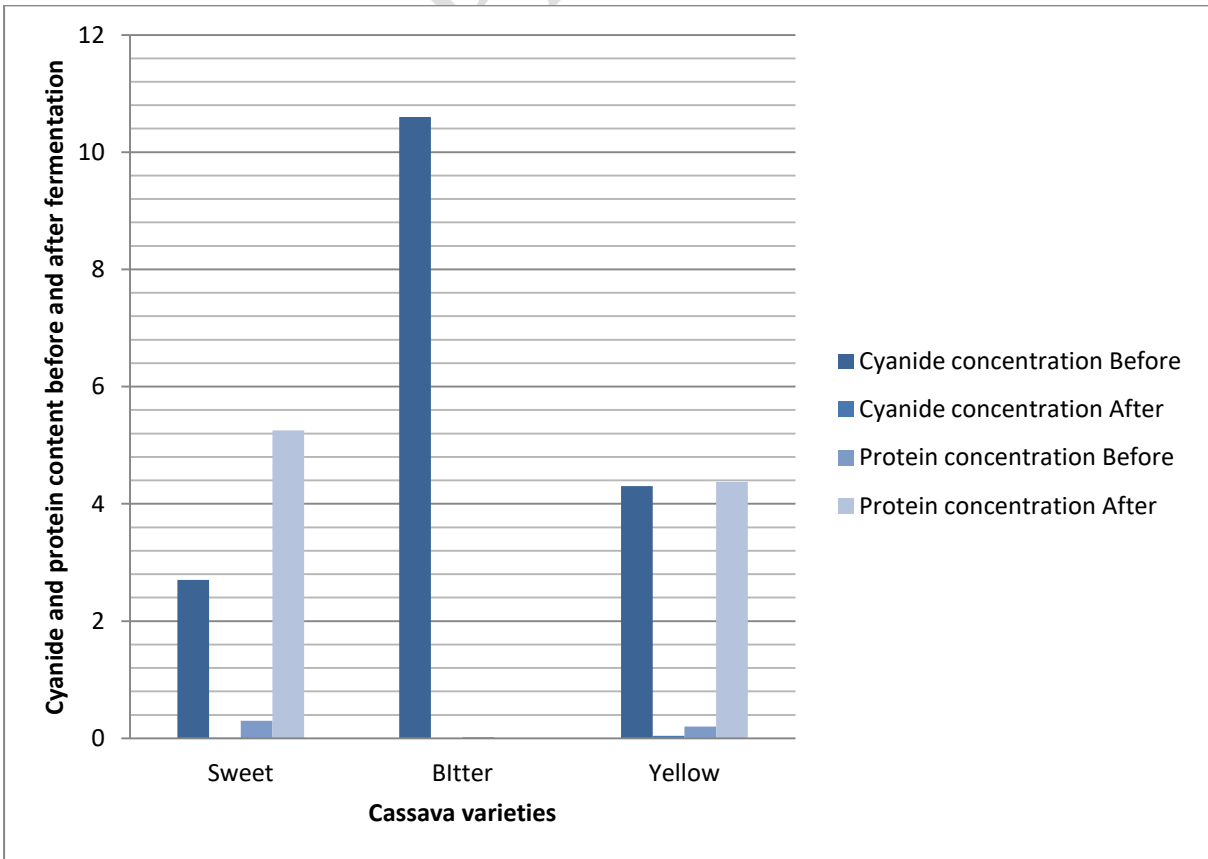


Figure 4: Cyanide and Protein content of the Fufu mash.

For sweet and yellow varieties, cyanide decreased, but % protein increased with retting time. For bitter variety, these parameters were not determined (ND) since it did not ferment.

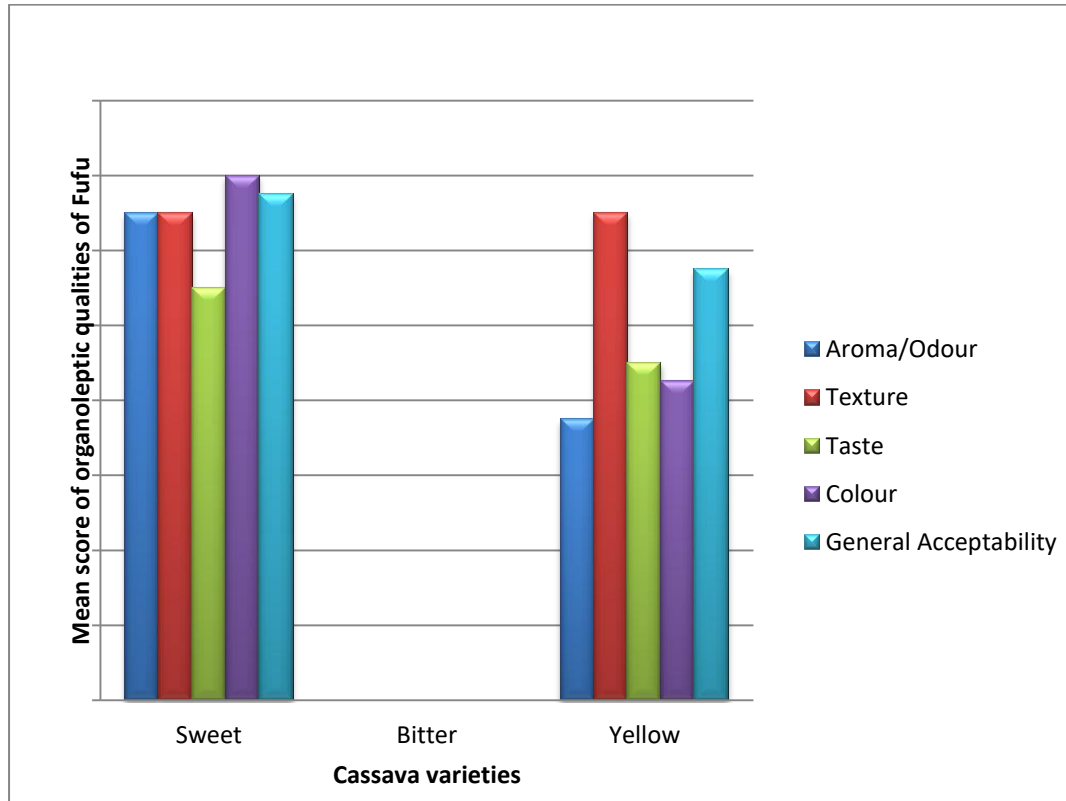


Figure 5: Mean scores of the Organoleptic qualities of the Fufu Samples.

For the sweet cassava variety, the mean scores of the organoleptic qualities are as follows: Aroma/Odour (6.5), Texture (6.5), Taste (5.5), Colour (7.0) and General acceptance (6.75). For yellow cassava variety, the mean scores of the organoleptic qualities are as follows: Aroma/Odour (3.75), Texture (6.5), Taste (4.5), Colour (4.25) and General acceptance (5.75). All the organoleptic parameters determined in the sweet cassava variety had higher acceptance except the texture which was the same with that of the yellow cassava variety (no significance difference). For the bitter cassava variety, these parameters were not determined because it has zero acceptance.

DISCUSSION

The study on the determination of starter organisms was carried out to detect the organisms that initiated retting and enhanced softening of the cassava tubers, reduced potential toxic cyanogenic glycosides in the tubers [20]. Microorganisms identified from the retting water included: *Bacillus subtilis*, *Escherichia coli*, *Staphylococcus aureus*, *Klebsiella aerogenes*, *Pseudomonas aeruginosa*, *Candida spp*, *Saccharomyces cerevisiae* and *Aspergillus spp*. Isolation of these organisms from retting water of cassava for fufu and their involvement in retting the cassava as starter organisms in this study agrees with the report of (31).

These number of bacteria and fungi from the retting water evident the involvement of multi-strain (mixed strain) starter organisms. This finding supports the report of Norman, [19] Isolation of *Saccharomyces cerevisiae*, *Aspergillus spp.* and *Candida spp* from the work as starter organisms, agrees with the report of [18, 24, 19, 25]. Daily counts of these organisms increased as retting progressed.

High multiplication of coliform during the earlier and middle days indicates mixed starter strain fermentation. This is in line with the reports of Fagbemi, [14]. Increased *Candida spp.* counts in the later stages of retting may be due to increased acidity of the retting water which favoured only the growth of fungi. The decreased bacterial and increased fungal loads observed in the retting water from sweet cassava variety can be attributed to low pH values which increased the acidity of the medium inhibiting bacterial growth, but enhanced fungal multiplication. This supports the findings of Kiyoshi and Tomoko, [26]. The fast retting of sweet white and yellow varieties may be attributed to reduction in their cyanide contents and increased protein content during fermentation process. The inability of the bitter variety to ferment can be due to high cyanide content of the tubers. This was also observed by Umeh and Odibo, [27].

Sweet white variety retted on the second day, whereas yellow variety retted completely on the fourth day, but bitter variety did not ret even on the fifth day. It can be stated that the starter organisms had greater impact on the retting ability of sweet white and yellow varieties, without effect on the bitter variety. On the other hand they function better under reduced cyanide than in high cyanogenic medium. So, these multi (mixed) starter organisms can only be applied in retting sweet white and yellow varieties and not bitter variety.

Conclusion

Based on these findings from this study, it can be concluded that the Sweet white variety is preferred to others for fufu production and should be allowed to ret under controlled condition for four days in order to reduce the cyanogenic glycosides and increase the protein content which will reduce the risk of diseases such as acute cyanide intoxication, goiter and ataxia and eliminate objectionable odor. Further researches are in progress to look into the phyto-chemical and proximate qualities of these Cassava varieties to ascertain the reasons for the differences in their retting ability and look out for other starter strains that can enhance fermentation of the bitter variety.

COMPETING INTERESTS DISCLAIMER:

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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