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Isolation, Characterization and Identification of Fermentation Process for the Production of Fufu and Effects of Starter Culture Development.

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ABSTRACT

Cassava (*Manihot esculenta* Crantz) is for the production of a variety of west African foods and ranks fourth in the list of major crops in developing countries, after rice, wheat and maize. Fermented cassava product like fufu is an important staple food in many African home. Natural fermentation time is usually long resulting in slower acidification process and inconsistent nutritional composition of products which could be overcome with the use of starter culture. However, must available starter cultures are used for single food fermentation and are uneconomical. This necessitate the development of a starter culture for multiple related food product to reduce cost. Hence this study was design to produce a common starter culture for the production of fufu. Cassava varieties TMS 30572 and NR 8082 where obtain from the farm at Anambra state, peeled and fermented in the laboratory. Lactic acid bacteria (LAB) where isolated from the fermenting mash and identified phenotypically, Fufu is a popular food produced from cassava. Cassava may contain high level of linamarin, a cyanogenic glucoside, which in its natural state is toxic to man. Most of the facultatively heterofermentative rods where identified by phenotypic tests as presumptive *Lactobacillus planterium* group strains, which also compromised the most predominant bacteria (54.4% of strains) isolated in study. The next predominant group of Lactic acid bacteria (14.1 % of total isolates) consisted of obligately heterofermentative rods belonging either to the genus *Lactobacillus* or *Weisella* followed by the heterofermentative cocci (13.9 % of isolates) belonging to the genera *Weisella* or *Luconostoc.* Homofermentative cocci were also isolated (13.3% of isolates). Biochemical properties, antimicrobials, acidification and fermentation of the indigestible sugars were evaluated *invitro* for selection of potential starter strains. Therefore, some processing methods that can enhance the detoxification of cassava and lead to the improvement of the quality and hygienic safety of the food are vitally important for less toxic products to be obtained. Quality, safety, acceptability of traditional fermented foods may be improved through the use of starter cultures. There has been a trend recently to isolate wild- type strains from traditional products for use as starter cultures in food fermentation.

Keywords: Fermented cassava, food fermentation, Lactic acid bacteria (LAB), *Lactobacillus planterium, Weisella* or *Luconostoc*

INTRODUCTION

Cassava (*Manihot esculenta Crantz*) is Africans second-most important food staple in terms of per capital calories consumed, and a source of calories for two out of five Africans [1]. It is a 1-2m high shrub which, due to its high yields of starch roots on marginal land, is a major staple crop for over 500million people in

the developing world [2]. Cassava has the ability to grow in poor and acidic soils, which are often not suitable for other crops, and yield a harvest in time of drought when all other crops have failed for lack of water [3,4]. Despite these advantages cassava has four major draw backs which limits it's utilization as a food [5]. These are low energy density, low protein content, rapid post-harvest deterioration and potential cyanide toxicity [6]. The starchy roots are the main food source, and the processed leaves are protein-rich food in Africa and elsewhere [7]. The tubers are usually processed into a variety of products such as gari, akpu, (fufu) and chips that can be processed into flour for bread and biscuit [8]. Food and Agricultural Organization [9] stated, that although these cassava provide basic source of dietary energy but are also deficient in protein. Utilization of cassava as food has been greatly affected by the presence of cyanogenic glucosides (Linamrin). The cyanogenic glucosides are present in all parts of the plant, with possible exception of the seeds [10]. Bitter varieties which contains higher number of cyanogenic glucosides have to be processed to remove the toxic compounds before consumption, whereas sweet varieties which have low levels of cyanogenic glucosides can be eaten fresh. Despite this population which uses cassava as main staple food, mainly grow the bitter varieties due to their higher yields their resistance to insect and therefore rely on processing methods for detoxification.

Fermentation is a metabolic process converting carbohydrates to organics acids. It is a good technology used some centuries. It is appreciating for it may advantages in food science. Fermentation can increase protein and vitamins content and improve the balance of essential amino acids. Fermentation increase also the volatiles organic content and reduce anti- nutrient contents. Fermentation not only enhances detoxification, but may also improve the quality and hygienic safety of the food [11]. The presence of yeast and lactic bacteria complicates the control of the fermentation process and lead to the production of objectionable odours. Such problems have led to the development of several other processing techniques suitable for odorless akpu. During fermentation of akpu, lactic acid bacteria, yeast and other bacteria contribute significantly to starch breakdown, acidification, detoxification and flavor development [12]. Among the fermented products of cassava, Cassava akpu is one of the favorites consumed in many parts of West Africa countries [13]. Cassava akpu is produced, sold and eaten in Nigeria and other African countries without any formal regulations or certification and this necessitate microbiological studies in other to ascertain its consumption and quality. Cassava akpu is a food made from soaked fermented cassava. Akpu is a popular cassava food found in several African countries. The alternative names of akpu are foufou, foofoo, fulful, fufu, utim, farine, yakayeke, agbalima according to Oyewole and Yemis [14]. Akpu is traditionally produced and marked as a wet, pasty food product. For the production of akpu, the preliminary operations units are similar to the one of garri. Cassava roots are then peeled, washed, cut into thick chunks of 20cm long and soaked in water contained in earthen ware pots or in a slow flowing stream. The fermentation takes about 4-5days. During this period, the cassava roots ferment and soften, releasing HNC into the soaked water. A characteristics flavor or retted cassava meal also is produced. The retted roots are disintegrated in clean water, sieved and the starchy particles that go through the sieve are allowed to settle for about 3 to 4 hours. The water is decanted while the sediment is packed into a cloth bag tied squeezed and subjected to a heavy pressure to expel excess water. The resulting meal is rolled into balls and cooked in boiling water for about 30 to 40minutes two consecutive times. The balls are boiled in water and the soft dough is produced [15, 16, 17,18]. The cooked mass is pounded in a mortar with

a pestle to produce a paste, akpu that can be eaten with sauce, soups or stew. Akpu is also sold to consumers in wet form,(uncooked) in small units packaged (plastic or polypropylene bags) or in ready to eat cooked form.

Statement of the problems

Lactic acid bacteria (LAB) are industrially important group of microorganisms employed in food fermentation and well known for their healthy and nutritional benefits [19]. There is an increased interest in the use of LAB in food preservation because of their safe association with human fermented foods and stability [20]. They are commonly found in foods and feeds and they are accepted as generally safe products for human consumption. Advantages of these fermented products include enhanced nutritional value, digestibility, therapeutic benefits, and safety against pathogens.

Akpu is an acid-fermented cassava product traditionally produced and consumed in Nigeria and other West African countries using different method of fermentation.

It is important to know which process is vital, healthy and economic for future application and hence it is necessary that microbiological study is varied out to evaluate its microbial content to validate its consumption safety and quality.

Scope of Study

The study is an investigation /Experimental research that is limited to two (2) cassava variety (white and yellow). The samples were collected from Urianiudo Ichida, Anambra State identified as TMS-30572(yellow cassava) and NR-8082(White cassava) using cassava seed tracker (seedtracker.org).

The research work expands on the review of past related works, methods of analysis and description, sample collection and process methods, Evaluation, observation detailing and comparison.

Materials and Methods

Period of Study

This study was conducted from 7th Nov to 19th Dec, 2020.

Study Area

UliIhiala Local Government area from Anambra State.

Source of Materials: Cassava tubers (One year old) of the specie TMS 30572, and NR 8082 were harvested from the farm at the Anambra State. Samples were collected and immediately transported to the Microbiology laboratory for processing within 24 hours for microbiological analysis.

Culture media, chemicals and reagents used was obtained from the Applied Microbiology Laboratory of the institution and were of analytical grade.

Methods of Akpu Production: The method of Oyewole and Ogundele [21] was used to produce wet akpu mash in the laboratory. The tubers after harvest were peeled, cut into cylindrical portions (4-7 cm long) and washed with tap water. They were washed again and dipped in 96% ethanol for 5 min for surface sterilization. This was then inoculated with different inoculate (Omo, Bitter-leaf, yeast). The control was allowed to ferment naturally. Three kg (3 kg) of the peeled cut tubers were soaked in 5liters of water for 4 days using plastic buckets with lid.

Sample Collection and Analysis

Samples for microbial analyses were taken during the fermentation stages for Akpu (fufu). The fermentation stage lasted for 4 days and samples were taken daily from the commencement of fermentation. Samples of fermenting cassava water and mash were collected in sterile 100ml conical flask and wide mouthed bottles respectively.

Total titratable acidity of fermenting cassava mash

Titratable acidity was determined using the standard titration procedure for total titratable acidity (TTA) described by [22]. Ten gram (10 g) of the fermenting mash was mixed with 90 mL sterile distilled water and homogenized. The mixture was filtered through Whatman Filter paper (No.1) and the filtrate titrated against 1M NaOH using 1% phenolphthalein as indicator. Acid equivalent is the amount of NaOH consumed in mL and each mL of

1M NaOH is equivalent to 90.08 mg of lactic acid.

Physicochemical analysis

The solution was analyzed for pH using Association of Official and Analytical Chemists [23] method.

Determination of PH

For pH measurements during the fermentation, 10 g of samples were added to 20 ml of distilled water and homogenized. The pH was measured every 6 h using a portable pH meter.

The pH meter (JENWAY 4510) was used to check the pH of the fermented broth and before fermentation. The pH of the solution was read and recorded for each day. The pH was done in triplicates and the mean recorded. This was done before fermentation and each day during 4days of fermentation.

Method of Microbiological Analysis

Determination of the Retting Ability of the Tubers:

The retting waters and tubers were monitored daily for retting ability and the presence of microbial flora.

-The retting ability of the tubers will be determined manually using the method of Umeh and Odibo [24].

-After retting the tubers were washed, mashed in clean water and sieved remove the fibers and the vascular bundles. The mixture was allowed to settle and excess water decanted. The wet akpu mash was transferred into a clean jute bag and the remaining water pressed out [21].

Microbial Isolation

10g of the fermenting cassava Akpu sample mash (fermentation of water and the cassava mixture) was taken aseptically on a daily basis (0/6hours, 24hours, 48hours, 72hours and 96hours) fermentation period. Microbiological analyses were conducted immediately after sampling by suspending 10 g of sample in 90 ml of sterile normal saline, vortexed and further diluted in a 10-fold dilution series and 1 ml of suitable dilution was inoculated onto various culture media and the various culture media were incubated at room temperature for 24-48hrs.

The following media were used as follows: Nutrient agar (NA) for general bacterial. Saboruad dextrose agar (SDA) general molds. Malt extract agar and potato Dextrose agar was used to isolate any yeast present during fermentation.

De Man Rogosa Sharpe (MRS) media were suited for isolating different lactic acid bacteria (LAB) groups and were thus used to obtain the greatest diversity of LAB associated with the fermentation., inoculum was spread onto agar, the plates were incubated aerobically at 30°C for 48 h.

Several streaking was done to obtain pure strains of the organisms on the same medium, after obtaining the pure strains of various organisms they were then sub-cultured into slanted bijou bottles. The pour plate method was used to determine the microbial counts in the retting water.

During isolation, the plates were observed and examined for changes in bacterial growth every 24hours interval during the fermentation periods. The different colonies in the media used were picked and sub-cultured each day during the fermentation period of 4/5days. The different colonies were labeled and examined for identification. The colonies that were seen during the ferrmentation were recorded. The microbial load was also recorded from the different media used.

Determination of Fungal succession at 24hours intervals

The different colonies on the plate were examined based on shapes (colony form) and sizes. The margin, color and elevation of the colony were noted.

Cultural Characteristics

Colonies developing on inoculated media were observed for pigmentation, elevation, consistency, colony surface and edges/margin.

Identification and Characterization of Isolates.

Characterization and identification of the bacterial isolates was carried out as stipulated by [25]. For isolation and identification of potential starter strains, colonies were randomly picked from the plates with the highest dilutions and

purified by streaking onto agar. Picking the bacteria from plates of the highest dilution ensured that the most predominant bacteria associated with the fermentation, that is, those occurring in highest numbers, were isolated.

Biochemical Identification

Bacteria and fungi present in the sample were identified using standard laboratory procedures which include Gram's staining, catalase test and sugar fermentation test. e.t.c Yeast strains were characterized by cell morphology.

1. Gram Staining

Carefully cleaned slides were properly labeled. A loopful of a 24hr culture of the organism was collected and smeared on the slide. It was then fixed by passing the smeared slide quickly through the flame so as not to scorch. The heat-fixed film of the organism in question was then immersed with crystal violet reagent for 60seconds after which it was washed with a gentle stream of tap water. It was then immersed in iodine mordent for 60seconds and again washed with gentle stream of tap water and allowed to dry. 95%(v/v) ethanol was then used to decolorize the crystal violet for 30seconds after which the film was counter stained with safranine red for 10 seconds and washed with water until no color appeared on the effluent. The slide was allowed to dry and then viewed under a microscope using oil immersion. Gram positive bacteria appeared purple while gram negative appeared pink.

2. Catalase

A loopful of the organisms from the nutrient agar plate was made on a clean slide and a few drops of hydrogen peroxide [H_20_2] were placed on it. The presence or absence of effervescence indicated a positive [+] or a negative [-] result respectively

3. H_2S Production

A tube of sterile peptone water was inoculated with the organism. A strip of lead acetate paper was placed at the top of the tube and held in place with a cotton- wool plug. The tubes were incubated for 48hrs and examined for blackening of paper. Blackening indicates positive otherwise is negative.

4. Motility

A ring of Vaseline was made on a glass slide. A drop of the suspension of the organism was then placed at the center of a clean cover-slip using a Pasteur pipette. The slide was then inverted over the cover-slip, which adhere to the slide and both were quickly re-inverted top produce a hanging drop of the culture on the cover slip. Motility was then examined under low power objectives X40 motile and non-motile isolates were noted.

5. Methyl Red

Microorganisms taken from 18-24 hours culture and lightly inoculate into the methyl red VOGES-PROSKAUER broth (buffered peptone 7 g/l, glucose 5 g/l, dipotassium phosphate=5 g/l) and incubate aerobically for 24hrs. 1 ml of the broth is added to a clean test tube and the remaining is reincubated for additional 24 hrs. 2-3 drops of methylred indicator is added to the aliquot in the testube. A red colour shows a positive result.

6. Voges-Proskauer Test

Microorganisms taken from 18-24 hours culture and lightly inoculate into the methyl red voges-proskauer broth (buffered peptone 7 g/l, glucose 5 g/l, dipotassium phosphate 5 g/l) and incubate aerobically for 24 hrs. After 24hrs incubation, 2mls of the broth is added to a test tube and the rest is reincubated for additional 24 hrs. 6 drops of 5% alpha-naphthol was added and mixed well to aerate. 2 drops of 40% potassium hydroxide is added and mixed well to aerate. The presence of a pink colour at the surface after 30 mins shows a positive result.

7. Indole

To a 4day peptone water culture of the organism is added about 0.5ml of xylene. The mixture was thoroughly shaken and allowed to stand for 30 minutes, a few drops of kovac's reagent was then added. The development of a rose pink colour showed a positive result while a yellow colour indicated a negative result.

8. Lactophenol Cotton Blue Mount (Yeasts)

A small drop of lactophenol cotton blue was placed in the center of a clean slide. A fragment of the yeast colony was removed with a probe or an inoculating needle and placed into the drop of the lactophenol cotton blue. A coverslip was then lowered gently on the preparation to mount it. The organism was then viewed under low power objective (x40) to observe the morphology of the yeast isolates.

9. Sugar Fermentation Test

Durham tube was placed in an inverted position in a bijou bottle containing 1% solution of the differential sugar (namely glucose, maltose, mannitol, fructose e.tc) in 1% peptone water with bromothymol blue (0.01 g/l) as indicator before autoclaving at 121° C for 15minutes. After autoclaving, the medium was allowed to cool to 40°C and organisms were inoculated. The bottles were then incubated at 37°C for 48 hrs. At the end of the incubation period, the tubes were observed for gas formation and acid production. The presence of air space in the durham tubes indicate gas formation. On the other hand, change in colour of the medium from green to yellow indicates acid production otherwise is negative (no acid production)

10. Starch Hydrolysis

Bacterial and yeast cultures were screened for amylolytic activity by starch hydrolysis test on starch agar plate. The pure isolated colonies were streaked on starch agar plates with starch as the only carbon source. After incubation at 37°C for 24hrs, the individual plates were flooded with Gram's iodine to produce a deep blue colored starch-iodine complex. In the zone of degradation, no blue color was formed and this is the basis for the detection and screening of amylase producing organisms. The amylase producers displaying maximum diameter of zone of clearance, were noted.

11. Acid production for yeast and LAB bacteria

The LAB test strains (1% of an overnight culture) were inoculated into MRS broth (pH 6.2 after autoclaving) and grown aerobically at 30°C. Acid production was determined by measuring the pH of the culture after 24 and 48 h. MRS broth medium was prepared from a single batch which was pH adjusted and then dispensed into tubes of 10 ml each before autoclaving [26].Acid production was not assessed for the yeast strains.

Results and Discussion

The fermentation of cassava roots, allows softening for further processing by *Bacillus subtilis, Staphylococcus epidemidis, Saccharomyces cerevisiae, Candida tropicalis* and reduction of potentially toxic cyanogenic glucosides present [27,28].

Clearly, it is evident that production of cassava fufu is initially mediated by a diverse micro flora, which eventually is dominated by the lactic acid bacteria. This pattern of microbial succession is general feature of fermenting plant materials as noted by [29, 30]. Table 1 show the microbial count at different days of retting. On the first day, the least bacteria count was on nutrient agar which is $1.07x10^6$ cfu/ml and on the 4th day the count was $5.33x10^6$cfu/ml showing the increase in microbial count as the days increased. There was increase in 4th day of Sabourad agar (SDA) which is $7.13.x10^6$cfu/ml, while on the 4th day of lactic acid bacteria the microbial count was 7.oo $x10^6$cfu/ml showing that there was an increase in microbial count as days goes by. This is an agreement with the findings of [13]. The increase in counts may be as a result of favorable conditions which enable them to multiply [3]. The reduced pH at the end of the fermentation could be as a result of the metabolic breakdown of starch into different organic acid metabolites. The growth and succession pattern of these organisms were reported by [4], to be dependent on factors such as water activity, pH, substrate, temperature and so on, thus, the microbial as well as resulting physicochemical interactions eventually regulates the number and

types of microorganisms that survives to the end of the fermentation process.

Gradual increase in LAB count with increasing fermentation time in fufu up till the end of fermentation as observed was similar to the reports of [2,5]. This could either be due to increase in acidity or low oxygen tension which favours growth of facultative anaerobes in submerged fermentation. It was, however, not surprising that subsequent drop in pH could lead to increase in LAB count since it has been reported that LAB predominate at low pH of fermenting medium [3].

It could therefore be said that increase in the number of more acid-tolerant organisms which in turn creates an unfavourable environment for non acid-tolerant ones might be the reason for the decrease in number of non-lactics, whereas, their high counts at the onset of fermentation could be linked to the fermentation water, materials and handling. Yeast counts decreased throughout the fermentation process. The observed decrease after 48 hours of fermentation could be due to a more acidic environment, brought about by increasing number of Lactic Acid Bacteria.

It has also been established that it is important to isolate predominant strains from fermentation batches for starter development. The observation of 50% each of randomly selected organisms from each fermentation being identified as *Lactobacillus plantarum* which was also the most prevalent was in line with reports of numerous authors that confirmed the predominance of lactic acid bacteria especially *Lactobacillus plantarum* during cassava fermentation. Furthermore, *L. plantarum* has been shown to have less complex nutritional requirements when compared to other *Lactobacillus spp.* Screening of organisms for starter development during cassava fermentation involves the ability of the microorganisms to produce microbial enzymes (amylase, linamarase, pectinase) which are essential for starch hydrolysis, cyanide detoxification and tissue disintegration, the ability to rapidly acidify the fermentation process as well as production of antimicrobial compounds which antagonize unwanted pathogens [7,8] Screening of strains in this study for starter development showed all isolates to be negative to starch hydrolysis. Amylase activity during fermentation could then be said to induced or constitutive as well as from other micro flora. [19], also reported the isolation of only a few amylolytic LAB. All the screened strains produced linamarase, an enzyme which is responsible for detoxification. Several microorganisms including *Bacillus* sp., lactic acid bacteria, *Lactobacilli, Leuconostoc, Streptococci* and yeasts, *Aspergillus, Fusarium, Penicillium and Trichoderma* strains are known for their detoxification activities during cassava fermentation. It could however be ascertained that the selected screened isolates can hydrolyse cassava cyanogens through their linamarase production even though, an endogenous linamarase could be inherent in the cassava.

Good pH reduction (a fast lowering of the pH) is important to accelerate fermentation process as well as reduce the levels of contaminating microorganisms which can compete with the starters for nutrients [10] and also a critical factor in developing flavour and aroma of foods. The decrease in pH values during the fermentation of cassava roots resulted from the production of organic acids by lactic acid bacteria. It has been established that the preservative action of starter culture in food is being attributed to a wide range of metabolites produced during fermentation. However, various antimicrobial compound (lactic acid, diacetyl and hydrogen peroxide) concentrations as observed in this study might be as a result of being produced by different strains since, [11] linked production level and proportion to be dependent on strains, medium compounds and physical parameters. The ability of LAB to inhibit the pathogenic organisms, could to be due to the fact

that they produce certain antagonistic substance which interferes with the metabolic activities of such pathogens, thus, inhibiting their growth.

Furthermore, starter cultures have been shown to have significant effect on fermented foods [8] at the end of 72 hours fufu fermentation. This work is in agreement with the work of [17] observed that if fresh cassava tubers were peeled, washed, cut and rewashed with 70% ethanol and rinsed with sterile water, any of these organisms can be inoculated aseptically and the resultant wet Cassava fufu can be produced..

Additives like bitter leaf, omo, yeast and a control were used to ferment cassava tubers to produce wet fufu (akpu) mash. Table1 shows the daily pH, and titratable acidity of the fermented cassava tubers

Table1: Total microbial counts of cassava retting water

Media	Days	Natural white	Natural yellow	Bitterleaf yellow	Yeast white	Omo yellow	Yellow yeast
Nutrient	1	$1.07x10^6$	$1.50x10^6$	$1.75x10^6$	$1.89x10^6$	$2.0x10^6$	$1.65x10^6$
	2	$2.33x10^6$	$2.68x10^6$	$2.50x10^6$	$2.79x10^6$	$2.92x10^6$	$2.63x10^6$
	3	$3.81x10^6$	$3.60x10^6$	$3.83x10^6$	$3.74x10^6$	$4.09x10^6$	$3.97x10^6$
	4	$5.25x10^6$	$4.03x10^6$	$5.11x10^6$	$4.9x10^6$	$5.33x10^6$	$4.84x10^6$
SDA	1	ND	$1.72x10^6$	ND	$1.90x10^6$	$1.99x10^6$	$1.06x10^6$
	2	$3.4x10^6$	$3.96x10^6$	$4.06x10^6$	$4.22x10^6$	$3.76x10^6$	$3.40x10^6$
	3	$4.22x10^6$	$4.3x10^6$	$5.40x10^6$	$4.78x10^6$	$4.52x10^6$	$5.05x10^6$
	4	$4.46x10^6$	$5.07x10^6$	$5.68x10^6$	$6.74x10^6$	$7.13x10^6$	$6.4x10^6$
LAB	1	$2.52x10^6$	$2.31x10^6$	$2.83x10^6$	$2.92x10^6$	$2.99x10^6$	$2.86x10^6$
	2	$3.31x10^6$	3.9^5x10^6	$3.02x10^6$	$3.85x10^6$	$4.01x10^6$	$3.9x10^6$
	3	$4.53x10^6$	$4.79x10^6$	$4.50x10^6$	$4.61x10^6$	$4.79x10^6$	$4.32x10^6$
	4	$4.84x10^6$	$6.75x10^6$	$5.80x10^6$	$6.68x10^6$	$7.00x10^6$	$6.30x10^6$

Table 2: pH and Total Titratable Acidity values during the period of fermentation

Samples	Day 1		Day 2		Day 3		Day 4		Day 5	
	pH	**TTA**	**pH**	**TTA**	**pH**	**TTA**	**pH**	**TTA**	**pH**	**TTA**
Natural white	4.5	1.7	3.8	0.9	3.7	0.74	3.8	0.9	3.53	0.92
Natural yellow	4.7	1.1	4.0	1.5	3.9	1.5	3.9	1.9	4.2	1.83
Bitterleaf yellow	4.8	0.9	4.3	1.2	4.6	1.8	4.6	1.9	4.2	2.6
Yeast white	4.8	1.1	4.3	1.5	4.2	1.61	4.1	2.5	4.0	2.8
Omo yellow	4.7	1.3	4.1	1.6	4.3	0.9	4.4	1.7	4.9	1.5
Yellow yeast	5.1	1.1	5.3	1.8	5.4	0.7	5.2	1.4	5.1	1.9

Keyword

TTA = Total titratable acidity

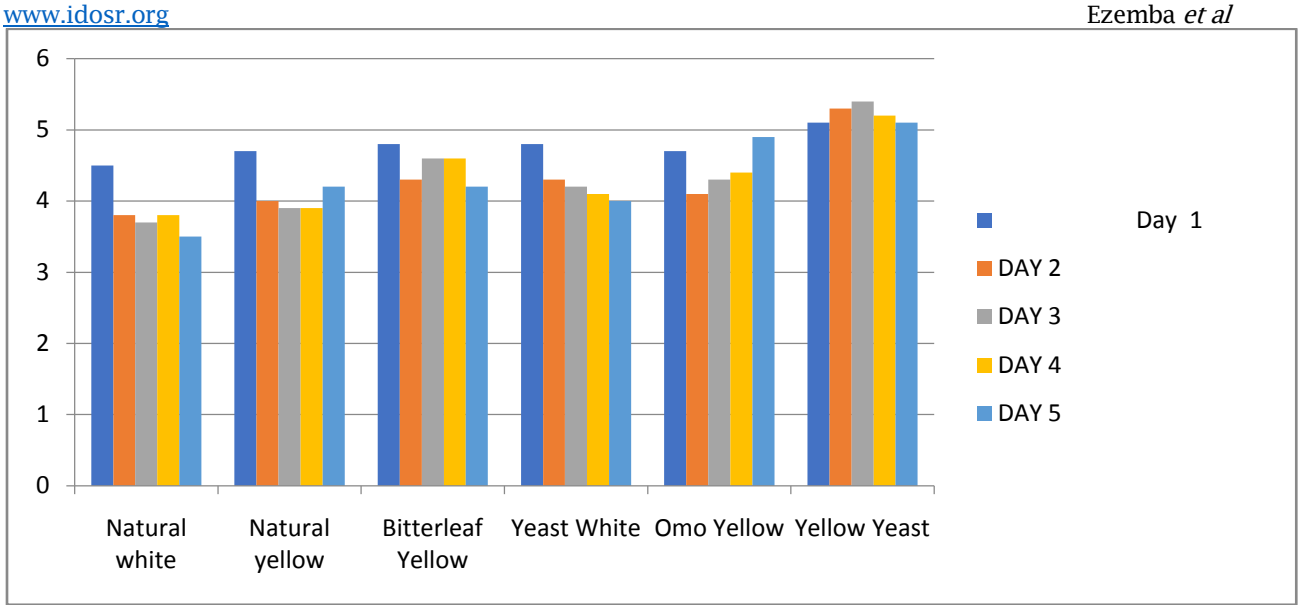

Figure 4: Bar chart illustrating pH range

Table 3: Appearance of cassava

Samples	1st day	2nd day	3rd day	4th day	5th day
1	clear	turbid	less foamy & turbid	less foamy & turbid	less foamy & turbid
2	clear	less turbid	less foamy & turbid	less foamy & turbid	less foamy & turbid
3	clear	foamy & turbid	less foamy& turbid	less foamy & turbid	less foamy & turbid
4	clear	foamy & turbid	foamy & turbid	foamy & turbid	foamy & turbid
5	clear	turbid	foamy & turbid	less foamy & turbid	less foamy& turbid
6	clear	foamy & turbid	foamy & turbid	less foamy & turbid	less foamy& turbid

Table 4: Morphological culture characteristics

Samples	SDA	LAB	MEA
Natural white	Fluffy whitish colony	Creamy mucoid colony and pointed colony	Smooth yellowish and whitish thread-like colony
Yellow yeast	Whitish thread-like mucoid colony and pointed colony	Pointed white colony, whitish thread –like colony and creamy mucoid colony	White big thread-like colony
Omo yellow	Whitish fluffy thread-like colony and mucoid colony	Whitish thread-like colony and pointed colony	Smooth creamy colony and small whitish colony
Natural white	Creamy mucoid fluffy colony, thread-like and pointed colony	Creamy mucoid colony and whitish pointed colony	White pointed colony and yellow pointed colony
Natural yellow	Mucoid and fluffy colony, creamy colony	Whitish thread-like colony	Blackish colony and yellowish colony
Bitter leaf yellow	Fluffy white mucoid colony and pointed colony	Mucoid small colony and small pointed colony	Smooth yellowish colony and small pointed yellowish colony

Table 5: Morphological and biochemical characteristics of bacterial isolates

Isolates	Colonymorphology	Gram staining	Motility	Methylred	Catalae	Starchhydrolysis	VP	Indole	H_2S	Glucose	Mannitol	Succrose	Lactose
Yellow yeast	Pointed white colony	+ rods in chains	-	+	-	+	**ND**	+	+	+	+	+	+
Natural yellow	Whitish thread-like colony	+ long rods	-	+	-	+	-	+	+	+	-	+	-
Bitterleaf yellow	Creamy mucoid colony	+ longthread-like colony	-	+	-	+	-	+	+	+	+	+	-
Yeast white	Smooth white colony	+ rods	-	+	-	+	-	+	+	+	+	+	+
Natural white	Whitish raised colony	+ small rod	-	+	-	+	+	+	+	+	+	+	-
Omo yellow	Rough creamy colony	+ Cocci rod	-	+	+	+	-	+	-	+	+	+	-

Keywords: ND = Not determined

Fig 1: White cassava leaf

Fig 2: Yellow cassava leaf

Plate 1: Cassava fermentation in a plastic fermenter

Fig 3: Positive H_2S Biochemical test

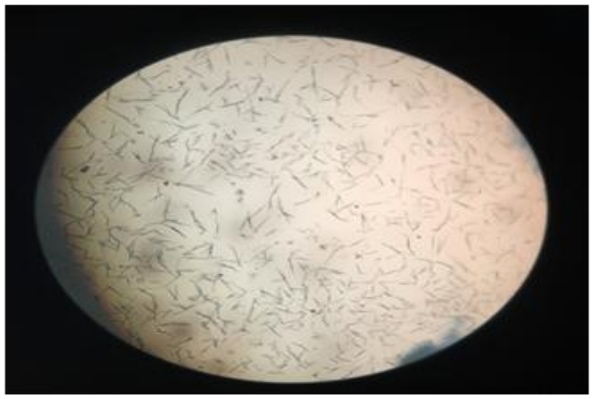

Plate 5: Microscopic view of Gram positive Lactic acid bacteria

CONCLUSION

The results of this study emphasize the independence of the preparation of starter culture on cassava lactic acid fermentation. They showed that whatever the variety of cassava roots used for the production of starter culture, the biochemical and microbial properties are approximately the same during the same period of fermentation and under the same conditions. In addition, this study revealed the progressive acidification of the pulp and the growth of diverse groups of microorganisms in starter culture which will determine the quality of cassava fermentation processing. The lactic acid fermentation of cassava is mainly bacterial. Among fermentative bacterial, LAB are predominant even if yeast and molds can be developed. However, high coliforms content in fermented cassava could contribute a health hazard to the consumer and could also act as potential spoilage agent of fermented cassava foods. So such starter with high level of coliforms could not be used as starter. The process could be improved by reduction of the coliforms content. The understanding of the preparation of a starter culture can be used for further development aimed to prepare a defined starter (defined micro flora) in order to more control the lactic acid fermentation processing so as to improve the quality of tropical traditional fermented cassava product. Although some isolated organisms (yeast and Lab) can also play an important role in industrial processes.

RECOMMENDATION

I recommend that further studies should be done on lactic acid as microorganism involved in the fermentation of cassava for akpu production. Also the waste water from the fermentation process, that causes environmental pollution could be used as a medium/source industrially.

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