Original Research Article

Effect of Microbial Inoculant on Physiological and Microbiological Properties of Cassava Fermentation Process and Fufu Produced

ABSTRACT

Aim: The effects of adding a microbiological inoculant on the physicochemical and microbiological properties during the fermentation process of cassava (*Manihot esculenta* Crantz) and on the sensory characteristics of the *fufu* produced using a local Bambili cassava variety were examined.

Study design: Completely Randomized Design was applied for this study.

Place and duration of study: Study was carried out at the Food Technology and Post-Harvest Programme Laboratory (FTPHL) of the Institute of Agricultural Research for Development (IRAD) Bambui, North West Region of Cameroon, between January and April 2020.

Methodology: Cassava was soaked in water and three treatments: 0%, 1% and 4% Light Matrix Organics (LMO) added to the water. The fixed submerged fermentation was used. Water samples were randomly collected daily and analysed for pH, temperature, Titratable Acidity (TA) and microbial counts. The experiment was carried out in triplicates, varying temperature each time (35^oC in trial 1, 32^oC in trial 2 and 21^oC in trial 3). Sensory evaluation was carried out on the fufu produced.

Results: Temperature, pH, TA and microbial counts followed the same trend in all 3 treatments. TA increased from 0.01-0.15%, while pH decreased from 6.9-4.85. Temperature increased then dropped at the end of the experiment. Total bacterial counts increased from $\log_{10}$ (3.49) to $\log_{10}$ (6.9) $CFUml^{-1}$. Yeasts and moulds increased from $\log_{10}$ (7.15) $CFUml^{-1}$ and then dropped to $\log_{10}$ (5.48) $CFUml^{-1}$ at the end of the experiment. Coliforms decreased from $\log_{10}$ (4.66) $CFUml^{-1}$ to $\log_{10}$ (3.30) $CFUml^{-1}$. The above parameters did not vary significantly ($p<0.05$) within treatments. Also, soaking temperature affected the duration of fermentation, hence the finished product. The natural fermentation process was preferred for colour, taste and flavour while the 1% LMO sample was preferred for texture.

Conclusion: Under good hygienic conditions; water fufu produced by natural fermentation will be of good quality for consumption.

Keywords: *Manihot esculenta*, fermentation, physicochemical, microbiological, sensory, microbial inoculant.

1. INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is the main staple food crop in the world. Its production is ranked sixth after maize, rice, wheat, potatoes, and soybeans [1]. It plays a major role in efforts to alleviate the African food crisis because of its efficient production of food energy, year-round availability, tolerance to extreme stress conditions, and suitability to present farming and food systems in Africa [2]. Physiological

deterioration of fresh cassava roots occurs 2-3 days after harvesting followed by microbial deterioration 3-5 days later [3]. Earlier studies state that cassava should be processed immediately after harvesting to avoid loss in nutritive quality [4]. Fermentation plays a great role in cassava processing, as it brings about the release of the enzyme linamarase from the plant tissues. This enzyme is involved in the breakdown of linamarin and lotaustralin (cyanogenic glucosides), which release Hydrogen Cyanides (HCN) and thus detoxify the product [5].

In Cameroon, cassava is an important root crop cultivated all over the country, most especially in the Centre, North West and South West regions, with a yearly production estimated at 2.3 metric tons [6]. The crop is mostly processed and consumed as garri, water-fufu, starch, cassava dough and boboloh. Other products include; mitumba, akara banana and bread made from cassava flour, cassava starch, and cassava spaghetti, among others. There is no limit to what can be done with cassava. More than two-thirds of the total cassava produced is used as food for humans, with a lesser amount being used for animal feed and industrial purposes. Pellets and alcohol for biofuel are also produced from cassava fermentation [7]. The cassava leaves are also consumed as a vegetable by humans and fodder for animals. Cassava contributes 76% of the total weight of starchy foods consumed in Cameroon and it is the third largest source of carbohydrates for human food in the world. Among the starchy staples, cassava is the cheapest source of calories for both human nutrition and animal feeding [8].

Today, microbial fermentation plays a vital role in food production. Some fermentation takes place with the help of laboratory-grown microbial inoculants. Cheeses for example are made by adding specific cultures of bacteria to milk at various stages of production [9]. Sometimes the fermentation relies on naturally occurring microorganisms in the food. Such is the case with cassava fermentation to produce fufu. Studies have been carried out on the microbial quality of products obtained from cassava fermentation [10]. These studies revealed that *Staphylococcus*, *Escherichia coli* and *Aspergillus* sp were the most common microorganisms found in fermented cassava products. Earlier studies carried out on the microbial diversity in the cooked fufu emphasized the need for improvement and maintenance of good hygienic practices by food handlers and food vendors. The biochemical and extracellular enzyme activities accompanying the fermentation process and the physiochemical and sensory analyses of cassava fermented fufu have been carried out. However, little has been done concerning the improvement of this product using probiotics.

Scarcely will one attend an occasion these days in Cameroon without water fufu and eru as one of the main meals. Many who would have loved to be part of this dish are not comfortable with some unfavourable characteristics of fufu such as poor texture, colour, smell and taste. Some consumers even complain of some malaise they get when they consume these products. Many, however, attribute these unpleasant characteristics to the fermentation process since they claim that the unfermented products of cassava do not possess these characteristics. Therefore, this study which was designed to examine the fermentation process of cassava using probiotics as a microbial inoculant was intended to improve the quality of fufu produced, so that this product could be loved by all and consumed without fear.

2. MATERIALS AND METHODS

The probiotic used in this study is Light Matrix Organics, which is a brownish liquid, with a pH of about 3.5, an acidity of 0.82% Lactic acid, and 100% soluble in water in any proportion, not viscous and smells like “corn beer” or fermented. It is translucent in colour and composed of a cocktail of rods, yeasts, cocci and other bacteria in the ratio 1:1:5:2:1.

The fermentation method used was the fixed submerged fermentation in which water remained unchanged throughout the fermentation process. The work was done in three trials, varying the temperature of the water used for soaking as follows; 35^{o}C in the first trial, 32^{o}C in the second trial and

room temperature (21^{o}C) in the third trial. Matured cassava tubers (two years old) of the local red variety were freshly harvested from the same farm few hours to processing time.

2.1. Preparation for Fermentation

This was achieved by a modification of the method described by [11]. Freshly harvested cassava roots were peeled and sorted. The disease-free ones were washed and reduced to small sizes of about 6 cm each using a knife. The cassava was filled into nine bottles of volume 3l each, which were previously sterilized by boiling and weighed. Water that had been boiled and allowed to cool to the required temperature needed per replication was used to soak the cassava. The water was boiled to get rid of any microorganisms that could have been present in the water. One percent (1%) of the microbial inoculant solution was prepared by measuring 10 ml starter culture into 1000 ml of the sterilized water. This was then poured into three of the nine bottles. These bottles were labelled T1. In the same way, 4% microbial inoculant solution was prepared by measuring 40 ml of the microbial inoculant into 1000 ml of water. This was used to fill three other bottles which were labelled T2. The three bottles remaining were left with no inoculant added. These were labelled T0 (control). Approximately 1755 g of tubers were steeped in 1505 ml of sterilized water in each previously sterilized bottle. All these were randomly placed in two coolers at room temperature (21±2^{o}C).

2.2. Analysis of Physicochemical Properties

Measurement of Temperature

The temperature was measured each day by dipping a food thermometer in each bottle and readings taken, making sure that the thermometer is sterilized before being dipped into the next bottle. The containers were observed, and readings taken throughout the fermentation process [12].

Measurement of pH

Ten (10) ml of each sample was collected each day and pH readings were taken using a pH meter (Hanna H198150 Glp pH/orp meter). This was done for all three treatments and the average pH per treatment was recorded [11].

Determination of Titratable Acidity

This was done according to the method of [11], where ten millilitres (10 ml) of solution from each container was pipetted into a beaker and 5 drops of Phenolphthalein indicator were added to each beaker using a dropper and burette. The volume of NaOH used was recorded as the titre. This procedure was repeated 3 times and the average of each three titre values was taken.

2.3. Microbiological Analysis

During the fermentation process, water samples were collected from each of the three treatments T0, T1 and T2, one of each treatment selected at random each day. These samples were used for microbiological analysis to enumerate: Total bacteria count, Yeasts and moulds, and Coliforms following the method described by [13].

Fifteen universal bottles containing 9 ml of 0.1% peptone water were collected and placed in 3 groups for T0, T1, and T2. Each group of five bottles was labelled 10^{1} to 10^{5}. One millilitre of the inoculum was pipetted into the first 9 ml of 0.1% peptone and mixed gently (10^{1} dilutions). This serial dilution was made up of the10^{5} bottle.

Aliquots (1ml) from the resulting serial dilutions were placed in a sterile petri dish using the pour plate technique in duplicate. The standard plate count agar (SPCA) (product of CondaPronadisa) was used for the determination of bacteria, incubated at 32^{0}C for 2-3 days while potato dextrose agar (PDA) (product of Liofilchem) was used for the determination of yeasts and moulds, incubated at 30^{0}C for 2-5 days and

violet red bile agar (VRBA) (OXOID) was used for the determination of coliforms, incubated at 37^0C for one (1) day. After incubation, only plates containing between 30-300 colonies were counted using an electric colony counter (Gallenkamp), assisted by a bold marker. Colonies counted were expressed in CFU/g.

2.4. Washing the Fufu

A sterile rod was driven into each bottle daily to access the softness of the cassava. When the cassava was soft, it was sieved using a sterile aluminium sieve and sterilized water to separate fibrous material from the fufu mash. The mash was allowed to settle after which each sample was poured into muslin clothes and allowed to drain. The product was then preserved in hermetically sealed cellophane bags. The sample which was soaked with water at 21^0C showed no sign of getting soft on the 6^{th} day of fermentation. The cassava was thus drained, blended and the mash was sieved, allowed to drain, then packaged. [5].

2.5. Cooking the Fufu

Two and a half kilograms (2.5 kg) of samples T0, T1 and T2 were each mixed with 1500ml of distilled water and cooked for 20minutes in a standardized procedure common to all samples (using a gas cooker) to a solid firm consistency. A wooden spoon was used to mix the fufu thoroughly to avoid lumps formation. With the help of a saucer, the fufu was rounded into small balls, ready for consumption [5].

2.6. Sensory Evaluation

The cooked fufu (all three samples separately represented) and eru was shared to a panel of 30 members consisting of 10 IRAD workers and 20 postgraduate students who were familiar with cassava fufu. They examined the texture, colour, smell and taste of the fufu and then filled the forms handed to them. The test was based on a 9-point hedonic scale with 1 being the least desirable and 9 being the most desirable.

2.7. Statistical Analysis of Data

All data obtained were entered on Excel and analysed using SPSS (Statistical Package for Social Sciences) version 16. Results were expressed as the means (±standard deviation) of triplicate (n=3) determinations. Data were subjected to one-way Analysis of Variance (ANOVA), using the chi-square test at $P<0.05$ level of significance.

3. RESULTS and DISCUSSION

3.1. Observable Parameters

In the first (35^0C) and second (32^0C) trials, all 9 samples were soft by day 3. In the 3rd (21^0C) trial, all cassava samples did not get soft up to the 6^{th} day.

This clearly indicates that soaking temperature affects the soaking and softening period for cassava. There is thus a temperature range that favours cassava fermentation. This goes in line with [14], who reported that a temperature range of 30-35^0C was best for submerged fermentation. This temperature is important not only for sterilization but to kick start the fermentation process as relatively high temperatures (30-35^0C) favour the growth of mesophilic microorganisms which are necessary for fermentation to take place. This explains why cassava in trial 3 (soaked at room temperature of 19-23^0C) did not get soft. These results show that soaking at room temperature in the Western Highlands of

Cameroon do not agree with those of [15][16] in Nigeria, where the room temperatures are relatively higher ($26\pm1^{0}C$).

3.2. Physicochemical Parameters

3.2.1. Effect of Temperature

Temperature changes followed the same trend in all three trials. For all treatments, temperatures dropped on day one, and almost remained constant throughout the fermentation process. (Table 1).

[17] also reported that heat is not generated during the fermentation process.

Table 1: Effect of temperature during the fermentation of Cassava in 3 treatments with time.

Number of days	Temperature per treatment (^{0}C)		
	T0 (0% LMO)	T1 (1% LMO)	T2 (4% LMO)
0	35^a±0.00	35^a±0.00	35^a±0.00
1	23^c±0.00	23^a±0.00	23^c±0.00
2	24^b±0.00	24^b±0.00	24^b±0.00
3	23.5^c±0.00	23.5^c±0.00	23.5^c±0.00

Means with the same letter along a column are not significantly different. P= 0.05

3.2.2. Effect of pH

During cassava fermentation, pH decreased with time in all three treatments (Table 2). The trend was the same in all three trials.

Cassava was soaked using water of pH near neutrality (6.9). Fermentation, an anaerobic process, involves the breakdown of starch by facultative anaerobic microorganisms such as lactic acid bacteria (LAB). These LAB produce high amounts of lactic acid and very little acetic acid. This leads to a rapid drop in pH to around 4.9 as previously found during the preparation of cassava fermented products such as *foo-foo, "attieke"* and *lafun* [18]. So, whatever the treatment, the trend in acidities is the same during lactic acid fermentation. However, the lowest pH values of treatment 3 (4% LMO)-the most acidic medium could be due to an additional number of microorganisms found in LMO, into the medium which led to a more rapid breakdown of starch than in other treatments. The significant difference in pH between Day 0 and Day 1 (pH falling from 6.9 to 5.18) could be as a result of the maximum accumulation of lactic acid in the bottles during fermentation after 24 hours. At the pH of about 5, the cyanohydrins formed disintegrate to form HCN which could be evaporated later from the fermented cassava. Indeed, fermentation allowed the elimination of more than 90% of endogenous cyanide compounds in the cassava roots after 48 hours. After this time, the acidity of the medium has already been attained hence changes in pH values become insignificant after Day 2. These results agree with those of [15] who obtained a change in pH from 6.8 through 4.3 to 3.8 after 96 hours of fermentation.

Table 2: Effect of pH on Cassava fermentation with time (days)

Number of days	pH per treatment		
	T0 (0% LMO)	T1 (1% LMO)	T2 (4% LMO)
0	6.90^a ±0.15	6.88^a ±0.15	6.40^a±0.15
1	5.28^b ±0.15	5.18^b ±0.15	5.30^b±0.15
2	5.26^b ±0.15	5.00^b±0.15	5.27^b±0.15
3	4.88^b ±0.15	4.85^b±0.15	5.26^b±0.15

Means with the same letter along a column are not significantly different. P=0.05

3.2.3. Effect of Titratable Acidity

During the fermentation process, titratable acidity values increased with days in all treatments, from a steeping pH of 0.01 to 0.15 by the end of the fermentation process (Table 3). Statistical analysis, however, showed that TA values did not vary significantly (p=0.1658) with days.

Titratable acidity (TA) is the total amount of acid [H^+] in the medium. The increase in TA within the treatments is the result of the fermentation of the cassava by the natural flora of microorganisms in the medium. Treatment 3 (4% starter culture) having the highest concentration of microorganisms showed a much rise in TA (0.09%). Studies by [17] revealed that while the pH of the fermenting cassava roots decreased, TA increased. These drop in pH and increase in TA in the fermentation water could be attributed to the accumulation of some organic acids such as lactic and acetic acids throughout the cassava fermentation process.

Table 3: Effect of Titratable Acidity (TA) during the fermentation process of cassava in three treatments with time.

Number of days	Titratable Acidity per treatment (%LA)		
	T0	T1	T2
0	0.01[a]	0.02[a]	0.03[a]
1	0.02[a]	0.05[a]	0.06[a]
2	0.03[a]	0.07[a]	0.13[a]
3	0.05[a]	0.10[a]	0.15[a]

Means with the same letter along a column are not significantly different at P=0.05

3.3. Microbiological Parameters

3.3.1. Variation in Total Bacterial Counts (TBC).

Total bacteria counts increased progressively from 3.49±0.28 cfu/ml in treatment T0 on day zero to 6.90±0.34cfu/ml in treatment T2 on day 3. Statistical analysis showed a significant difference (p=0.0001) in total bacterial counts with days. The highest counts were recorded in treatment T2, while the least counts were recorded in treatment T0 (Table 4).

The concentration of microorganisms in the medium varies from one day to the next. It was normal for treatment T2 with highest concentration of inoculant to have higher counts due to the presence of bacteria in the LMO. Not all microorganisms can grow in the acidic medium. As the medium becomes more acidic, some microorganisms (acidophobes) like *Bacillus subtilis* which cannot withstand this environment die in the course of the fermentation process, while acidophiles such as yeasts and lactic acid bacterial thrive to the end of the experiment. From this reasoning, it could be said that the progressive rise in bacterial count could be due to the presence of lactic acid bacteria that are responsible for breaking- down starch to sugar during fermentation. The drop in bacterial count is in agreement with [17], who reported that the population of Bacillus sp. decreased drastically as fermentation progressed.

Table 4: Variation in TB counts during the fermentation of cassava in three treatments with time.

Number of days	Total Bacteria Counts per treatment (log_{10}cfu/ml)		
	T0 (0%LMO)	T1 (1%LMO)	T2 (4%LMO)
0	3.49^{a}±0.28	3.52^{a}±0.28	4.53^{b}±0.28
1	4.52^{b}±0.34	5.32^{c}±0.34	5.49^{c}±0.34
2	5.85^{c}±0.32	5.70^{c}±0.32	5.88^{c}±0.32
3	6.48^{d}±0.34	6.60^{d}±0.34	6.90^{d}±0.34

Means with the same letter along a column are not significantly different at P=0.05

3.3.2. Variation in Yeasts and Mould Counts

Yeasts and mould count increased progressively from day zero to day 2, then dropped on the third day of fermentation in all three treatments. Statistical analysis showed a significant difference (p=0.05). The highest counts were recorded in treatment T2, while the least counts were recorded in treatment T0 (Table 5).

Yeasts are important in cassava fermentation. The increase in yeasts and moulds on Day 2 could be due to a favourable environment (pH of approximately 5.17, titratable acidity of 0.08% and temperature of 24^{0}C) for growth. [14] affirmed that yeast cells can tolerate a pH of 4.0 to 8.5, with optimum pH between 4.0 and 6.0. A decrease in yeasts and moulds at the end of the fermentation process could be due to the depletion of substrates and bacteria competition.

Table 5: Variation in Yeast and Mould counts with time during the fermentation of cassava in three treatments.

Number of days	Yeast and Mould counts per treatment (log_{10}cfu/ml)		
	T0	T1	T2
0	3.78^{a}±0.42	5.28^{c}±0.42	5.34^{c}±0.42
1	4.00^{b}±0.50	5.60^{c}±0.50	6.54^{d}±0.50
2	5.15^{c}±0.44	6.30^{d}±0.44	7.15^{e}±0.44
3	4.30^{ab}±0.50	4.48^{b}±0.50	5.48^{c}±0.50

Means with the same letter along a column are not significantly different.

3.3.3. Variation in Coliform Counts

Coliform counts decreased progressively from day zero through day 3. This showed a significant difference (p=0.0003). Averagely, there were more coliforms in treatment T2 and least in treatment T0 (Table 6).

Coliforms in foods are an indication of possible contamination. It has been reported that the presence of coliforms in fermented cassava could constitute a health hazard to the consumer and could also act as a

potential spoilage agent of fermented cassava foods. So, improvements in hygienic conditions and in the selection of raw materials are required for *water-fufu* production.

Table 6: Variation in coliform counts during the fermentation of cassava in three treatments with time.

Number of days	Coliforms per treatment (log_{10}cfu/ml)		
	T0	T1	T2
0	4.08^{a}±0.35	4.66^{a}±0.35	4.52^{a}±0.35
1	3.50^{b}±0.35	3.59^{b}±0.35	4.22^{a}±0.35
2	3.44^{c}±0.35	3.49^{b}±0.35	4.00^{a}±0.35
3	3.30^{c}±0.35	3.35^{b}±0.35	3.48^{b}±0.35

Means with the same letter along a column are not significantly different.

3.4. Sensory Evaluation Results.

Panellists gave attributes to smell, taste, texture and colour. Treatment T0 recorded the highest scores for smell (8.35), taste (8.22) and colour (8.50) while treatment T1 recorded the highest score for texture (8.36) (Table 7).

Lactic and acetic acids released when starch is metabolized create a favourable environment for yeasts that lead to the characteristic odour of *fufu* [19]. The highest score for smell registered by treatment T0 could be attributed to the unpleasant fermented smell of LMO, which could possibly affect the *fufu* produced. This was confirmed as T2 with the highest concentration of LMO, scored least (7.08) for smell. Most panellists preferred the whitish colour of *water-fufu*. *Fufu* of any other colour is most often regarded to be of doubted quality. The brown colour of LMO must have affected the colour of the LMO-inoculated *fufu.* The result for taste might be accepted with doubts since the *fufu* was eaten with *eru,* which could influence the taste of the *fufu*. Panellists, however, could not have eaten the cooked *fufu* without a supplement. Preference of T2 for texture could lead to the conclusion that small amounts of LMO may be accepted to produce *fufu* with good texture.

Table 7: Attributes by variation T0, T1 and T2 in samples of the fermentation of Cassava in three trials at 35^{0}C, 32^{0}C and 21^{0}C.

Attribute	**T0**	**T1**	**T2**
Smell	8.35	8.20	7.08
Taste	8.22	7.53	5.71
Texture	8.28	8.36	7.99
Colour	8.50	8.13	6.53

CONCLUSION

The effect of the addition of 1% and 4% LMO, (a microbial inoculant), was compared with natural fermentation (no inoculant) to evaluate the effect of this inoculant on changes in the physicochemical and microbial properties during the fermentation process and the sensory characteristics of the *fufu* produced. Results obtained show that; The soaking temperature affects the duration of fermentation. Temperature, pH and titratable acidity follow similar trends irrespective of treatments. Microbial counts do not vary significantly from one treatment to another. Lactic acid bacteria which is responsible for breaking down cassava starch to sugar, produced an acidic condition that discouraged undesirable microorganisms but favours the growth of yeasts which presumably impart the characteristics flavour of naturally produced 'water fufu'. LMO does not affect the fermentation process, but slightly affects the quality of the finished product. The additional microorganisms found in LMO do not play any significant role in the fermentation process. Fermentation with LMO is seen to produce fufu that is brownish, with a scent. These attributes make the fufu unattractive to the consumer. Natural fermentation with no inoculant produces better fufu based on taste, smell and colour. Hence, natural fermentation under good hygienic conditions should be encouraged for water fufu production.

REFERENCES

[1] Reinhardt H. “Cassava cultivation and soil productivity,” in *Achieving sustainable cultivation of cassava*, 1st ed., 2017, p. 8.

[2] Okogbenin E. “Phenotypic approaches to drought in cassava: review,” *Front. Physiol.*2013; 10(4) :93, 2013.

[3] Naziri D, Quaye W, Siwoku B, Wanlapatit S, Viet T and Bennett B. “The diversity of postharvest losses in cassava value chains in selected developing countries,” journal of agriculture abd rural development in the tropics and subtropics.2014;115(2):111–123.

[4] Oboh G. “Nutrient enrichment of cassava peels using a mixed culture of Saccharomyces cerevisae and Lactobacillus spp solid media fermentation techniques,” *Electron. J. Biotechnol.*2006;9(1):46–49.

[5] Uyoh E, Ntui V and Udoma N. “Effect of local cassava fermentation methods on some physiochemical and sensory properties of fufu,” *Pakistan J. Nutr. 2009*;8(8):1123-1125.

[6] Hanna R. “Farmers Perception and Criteria for Cassava Variety Preference in Cameroon,”African study monographs.2013; 34(4): 221–234.

[7] Larsson S, Lockneus O, Xiong S and Samuelsson R. “Cassava Stem Powder as an Additive in Biomass Fuel Pellet,” Energy and fuels.2015.

[8] Tonukari N, “Cassava and the future of starch.,” *Biotechnol. issues Dev. Ctries.* 2004; 7(1):5-8.

[9] Marcelino JK. “Lactic Acid Bacteria as Starter-Cultures for Cheese Processing,” in *Past, Present and Future Developments*, 2013.

[10] Adebayo-Oyetoro A, Oyewole O, Obadina A, and Omemu M. “Microbiological Safety Assessment of Fermented Cassava Flour Lafun Available inOgun and Oyo States of Nigeria,” *Int. J. Food Sci.* 2013; 1–5.

[11] Braide WC, Azuwike and Adeleye SA, “The role of microorganisms in the production of some indigenous fermented foods in Nigeria,” *Int. J. Adv. Res. Biol. Sci.*2018;5:86–94.

[12] Luo G, Xie L, Zou Z, Zhou Q and Wang J. “Fermentative hydrogen production from cassava stillage by mixed anaerobic microflora : Effects of temperature and pH,” *Appl. Energy*.2010;87(12):3710–3717, 2010.

[13] Anihouvi VB, Sakyi-dawson E and Hounhouigan DJ. “Microbiological changes in naturally fermented cassava fish (Pseudotolithus sp .) for lanhouin production,”International journal of food microbiology.2007;116(6):287-291

[14] Elmer L, Mpoko B, Susan H, Clifford W, and Keith H. *Applications of Biotechnology to Traditional Fermented Foods*. Washington, D.C.: National Academy Press, 1992.

[15] Achi O and Akomas NS. “Comparative Assessment of fermentation Techniques in the Processing of Fufu, a Traditionally Fermented Cassava Product,” *Pakistan J. Nutr.*2006;5(3): 224–229.

[16] Oyeyinka SA, Adeloye AA.,. Olaomo O. O, and Kayitesi E. “Effect of fermentation time on physicochemical properties of starch extracted from cassava root,” *Food Biosci.*2019;100485:In Press

[17] Oyewole O and Odunfa S. “Microbiological studies on cassava fermentation for ‘lafun’ production,” *Food Microbiol.*1988;5:125–133.

[18] Omafuvbe B, Adigun A, Ogunsuyi J, and Asunmo A, “Microbial Diversity in Ready-To eat Fufu and Lafun-Fermented Cassava Products Sold in Ile-Ife, Nigeria,” *Res. J. Microbiol.*2007;2:831–837.

[19] Tanya AK, Darman D, Ejoh A, Mbahe R, and Hamidou, “Physiochemical and sensory analysis of fermented flour ‘Kumkum’ from three improved and one local cassava varieties in the Adamawa Province of Cameroon,” *Pakistan J. Nutr.*2006;5:355–358.