Microbiological assessment of fufu produced from Akoko area of Ondo State

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Abstract:- Fufu **is a fermented food from cassava tubers which when processed are hawked by the producers. Microbiological and physicochemical analyses were conducted on processed *fufu* samples obtained from six different communities in Akoko Area (Akungba, Ikare, Iwaro, Ayegunle, Supare and Oba) of Ondo State, Nigeria. The mean total aerobic bacterial counts, lactic acid bacteriai counts and the fungal counts of the samples ranged from $1.66x10^6$ cfu/g to $4.61x10^6$ cfu/g, $2.4 x10^6$ to $4.85 x10^6$ cfu/g and $1.5x10^3$ to $2.65x10^3$ cfu/g respectively. Bacteria isolated from the samples included *Staphylococcus aureus, Leuconostoc mesenteroides., Bacillus cereus, Escherichia coli, Streptococcus* sp.*, Lactobacillus plantarum* and *L. fermentum* while the fungi included *Candida albicans, Mucor mucedo, Aspergillus niger, A. flavus, Fusarium oxysporum, Rhizopus stolonifer, Saccharomyces cerevisiae* and *Penicillium chrysogenum*. The pH of the samples ranged from 3.9 to 5.0, the total titratable acidity ranged from 1.44% to 3.18% while the temperature ranged from 26^o C to 28^oC. The moisture contents of the *fufu* samples ranged from 55.02% to 73.16 %, while the carbohydrate and protein contents ranged from 16.65 to 31.27% and 1.66 to 3.80% respectively. Antinutrient analysis showed that phytate had the highest value (mean value of 67.76/100mg) while tannins had the lowest value (mean 2.00/100mg).The cyanide content ranged from 0.32mμ/100g to 0.42mμ/100g.The mineral content analysis revealed the abundance of magnesium and low level of iron in all the *fufu* samples. The detection of some pathogenic microorganisms in the *fufu* samples underscores the importance of clean production environment, proper handling methods, and good personal hygiene of *fufu* producers and retailers.**

Keywords: **microbial analysis, *fufu,* Akoko, food safety, proximate quality**

I. INTRODUCTION

Fermented foods are an intrinsic part of the diet of Nigerians and indeed all other places where the crop is grown in large quantities. The fermented foods are treasured as important dietary constituents in many developing countries because of their availability, easy production, keeping quality, local acceptability and relatively cost effectiveness (Adegbehingbe *et al.,*2017)). *Fufu* is one of the most consumed staple foods in Nigeria, especially in the Southern parts of the country.It is a fermented product from cassava(*Manihot esculenta*) tubers which has been estimated to provide around 70% of the daily calories needs of 50 million people in Nigeria (Oluwole *et al*., 2004). It is second to *garri* among the fermented cassava products in Nigerians particularly in the south east (Okafor *et al*., 1998).

*Fufu*comes as a wet mash or processed to dry powder which can be cooked and pounded or stirred in boiling water to form dough (Umeh and Odibo, 2013). Cooked *fufu* is usually sold in transparent polythene bags or sold in restaurants and taken with desired soup. It is sold in the wet form in some places (moisture about 50%) which renders it highly perishable (Oguntunde and Orishagbemi, 1991). The method of *fufu* preparation varies from locality to locality and this greatly affects the quality of the finished product (Okpokiri*et al*.,1985).The cooked *fufu* has a shelf life of about 72 hours and can be eaten while hot or cold (Omafuvbe, 2007).

Cassava products, like other food materials, have potentials for supporting the growth of both pathogenic and spoilage microorganisms. These microorganisms may be introduced directly during processing, handling, storage or hawking. These could invariably contribute to changes in texture, taste, appearance and smell, and reduction in the safety and acceptability of the food product.

Therefore, the objectives of this present study were to isolate and identify the microorganisms present in processed *fufu* from six communities in Akoko area, Ondo State, Nigeria, and to determine the proximate, anti-nutrient and mineral contents of the processed *fufu* samples.

II. MATERIALS AND METHODS

Collection of samples

Fufu samples were collected aseptically from the processors and sellers in 6 communities (Akungba, Ikare, Iwaro, Ayegunle, Supare and Oba) in Akoko area, Ondo State. The samples were collected in sterile containers and transported to the Microbiology Laboratory of Adekunle Ajasin University, Akungba-Akoko for analysis.

Production of fermented fufu flour

Cassava root tubers of about 12 months old were obtained from a local farm in Akungba-Akoko, Ondo State. One stage method of *fufu* preparation was used during this investigation. The cassava tubers were peeled and washed.

Three kilograms of the peeled tubers were sliced into cylindrical pieces and steeped in water for 96 hours. The resulting soft fermented cassava tubers were hand pulverized and sieved using sieve of about 1.00 mm aperture. The resulting mash was allowed to sediment for 12 hours before the top water was decanted. The sedimented mash was then poured into a jute bag to drain the water from the mash. The resulting wet product, *fufu*, was further dried in a single layer at 65^{o}C for 48 hours in a cabinet dryer. This dried *fufu* cake was then milled to powder.

For the production of unfermented cassava flour, cassava root tubers were peeled, cut into thin chips and washed properly to remove dirts. The chips were then dried in a cabinet dryer at 65^{o}C for 48 hours. The dried chips were then milled and sieved to get the unfermented cassava flour (Oyewole and Ogundele, 2001). The fermented and unfermented cassava flour samples served as controls.

Isolation and Identification of microorganisms from the fufu samples

Ten grams of each sample were transferred into 90ml of sterile distilled water, and then homogenized appropriately. One millilitre was aseptically transferred into 9 ml of sterile distilled water in a sterile test tube to make a factor of 10^{-1}. More tenfold dilutions were made till 10^{-6} dilutions.Pour plate technique was employed for the enumeration and isolation of the isolates.Nutrient Agarand de Man Rogosa Sharpe Agar were used for the isolation and enumeration of total bacteria and lactic acid bacteria in the samples at 37^{o}C and 35^{o}C for 24 hours and 48 hours respectively while Sabouraud Dextrose Agar for fungi at room temperature for 3 to 5 days. The representative isolates were later purified by repeated streaking on appropriate media and the pure cultures were stored on agar slants. The bacterial isolates and the fungal isolates were characterized and identified using their cultural and morphological characteristics as well as biochemical tests according to Ochei and Kolhatkar (2008) andAlexopoulous*et al*.(1996) respectively.

Physicochemical analysis of the fufu samples

Determination of pH and Total Titratable Acidity (TTA)

The pH and total titratable acidity of the samples were determined according to the methods described by Olutiola*et al.* (2000).

Proximate composition of the fufu samples

The moisture, ash, fat, and protein contents of the samples were determined according to methods of AOAC (2005). Total carbohydrate content was determined by difference.

Mineral and Anti-nutritional factor analysis

The mineral analysis and the determination of anti-nutrients (cyanide, saponin, oxalate, tannins, phytate, phenol) levels in the *fufu* samples were determined using methods of AOAC (2005).

III. RESULTS

Microorganisms isolated from the *fufu* samples were predominantly lactic acid bacteria and yeasts. The genera included *Lactobacillus* and *Leuconostoc* and *Saccharomyces cerevisiae*. However, aconsortium of microorganisms were isolated from grated unfermented cassava tuber which included the bacteria *Escherichia coli, Bacillus cereus, Staphylococcus aureus, Leuconostoc* sp, *Streptococcus* sp.and the fungi *Rhizopus stolonifer, Aspergillusniger* and *A. flavus* among others (Table 1).Other fungal isolates included *Penicillium chrysogenum, Candida albicans, Fusariumoxysporum,* and *Mucormucedo. Rhizopus stolonifer* and *A. niger* were only isolated from samples obtained from Iwaro and Supare Akoko.

The physicochemical properties of the unfermented, fermented and the *fufu* samples obtained from various communities are depicted in figures I, 2 and 3. The pH of the processed samples ranged from3.9 to 4.5, while that of the unfermented sample (control) was 5.1.The total titratable acidity of the samples ranged from2.44% to 3.18% and that of unfermented sample was 0.53%. The temperature of the unfermented and fermented ranged from 28^{o}C to 31^{o}C.

The total bacterial count was $3.43x10^{6}$cfu/g, Lactobacilli count was $3.9x10^{6}$cfu/g, and the fungal count was $2.27x10^{3}$cfu/g.The highest bacterial count of $5.3x10^{6}$cfu/g was observed in samples from Oba-Akoko while the least ($1.66x10^{6}$cfu/g) was observed in samples from Akungba-Akoko (figure 4). Furthermore, the highest fungal count of $2.65x10^{3}$cfu/g was obtained in Oba-Akoko samples with the least ($1.5x10^{3}$cfu/g) coming from Akungba-Akoko.

The results of the proximate analysis of the six processed *fufu*samples (figure 5). The moisture content of the samples ranged from 55.02% to 73.16%. The carbohydrate contents of the six samples were high while the crude protein, ash and crude fiber contents were low.The cyanide content of unfermented sample was higher when compare to other samples (figure 6). The cyanide content ranged from0.32µg/100g to 0.42 µg/100g. Phytate had the highest value while tannins had the lowest value in all the six samples. Phenol had a value that ranged between 7.05 mg/100g and 14.09 mg/100g. Magnesium contents of the samples were the most abundant mineral in both fermented and fermented which ranged from 156 to 165 mg/100gwhile ironcontents being the least and ranged from 0.03 to 1.42mg/100mg (figure 7).

Table 1: Microorganisms isolated from *fufu* samples

Sample code	*Bacillus cereus*	*Staphylococcus aureus*	*Leuconostoc mesenteroides*	*Streptococcus feabalis*	*Escherichia coli*	*Lactobacillus fermentum*	*L. plantarum*	*L. lactis*	*Saccharomyces cerevisiae*	*Aspergillus niger*	*A. flavus*	*Mucor mucedo*	*Rhizopus stolonifer*	*Fusarium oxysporum*	*Penicillum chrysogenum*	*Candida albicans*
Unfermented (Control)	+	+	-	+	+	-	-	-	-	+	-	-	+	-	-	-
Fermented (Control)	-	-	+	-	-	+	+	+	+	-	-	-	-	-	-	-
FAA	+	+	-	+	+	+	+	-	-	-	+	+	-	-	-	+
FKK	+	+	+	+	+	-	+	-	-	-	-	-	-	-	-	+
FWK	+	+	-	+	-	-	+	-	-	+	+	-	+	+	-	+
FYE	-	+	+	+	-	+	+	-	-	-	+	-	-	+	+	_
FSP	-	-	+	+	-	-	+	-	-	+	-	+	+	+	+	_
FFA	-	+	+	-	+	+	+	-	-	-	-	+	-	-	+	+

- : Negative, + : Positive,FKK= Ikare Akoko; FWK= Iwaro-Oka Akoko; FYE= Ayegunle Akoko; FSP= Supare Akoko;FFA=Oba Akoko

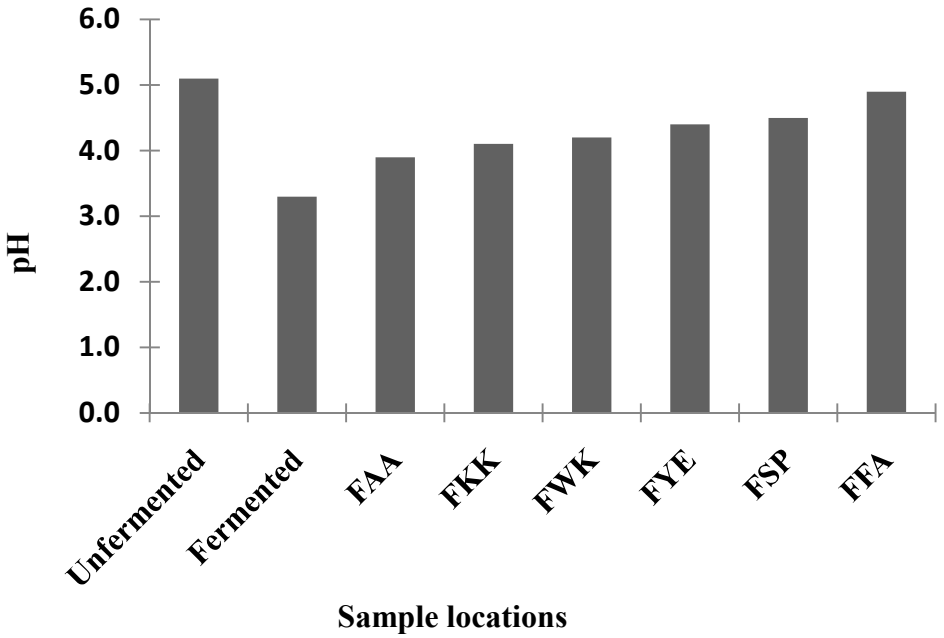

Figure 1: The pH of the *fufu* samples, FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE= Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko.

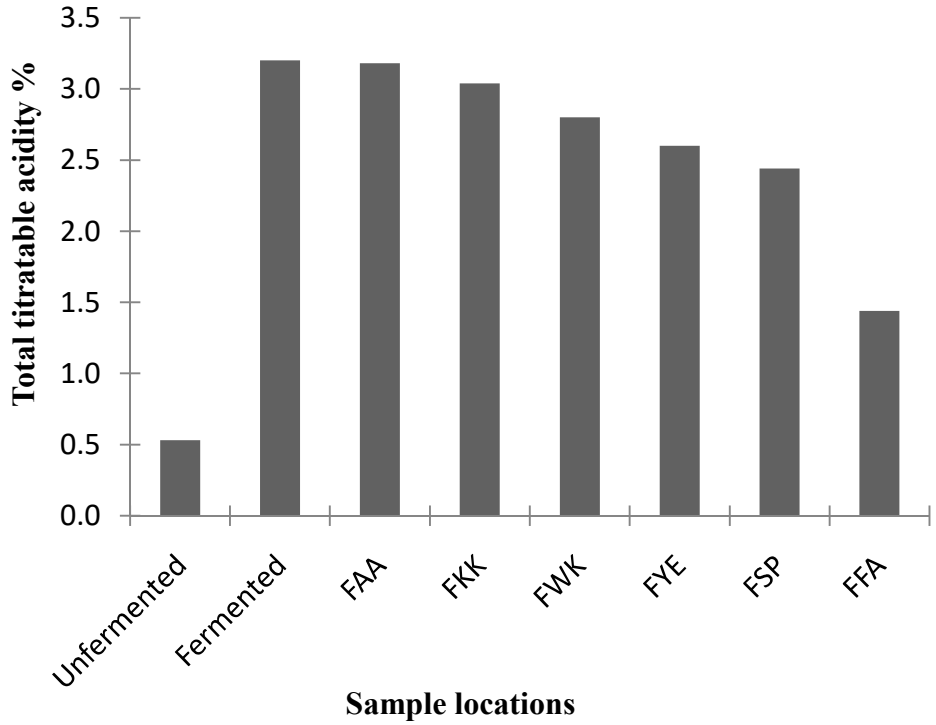

Figure 2: The total titratable acidity of the *fufu* samples, FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE= Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko.

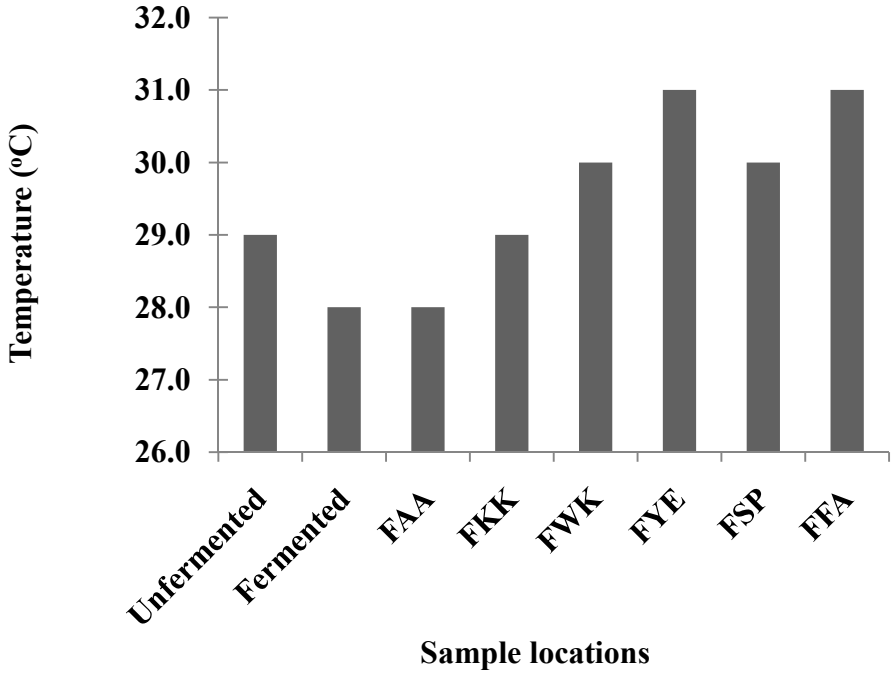

Figure 3: The temperature of the *fufu* samples, FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE= Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko

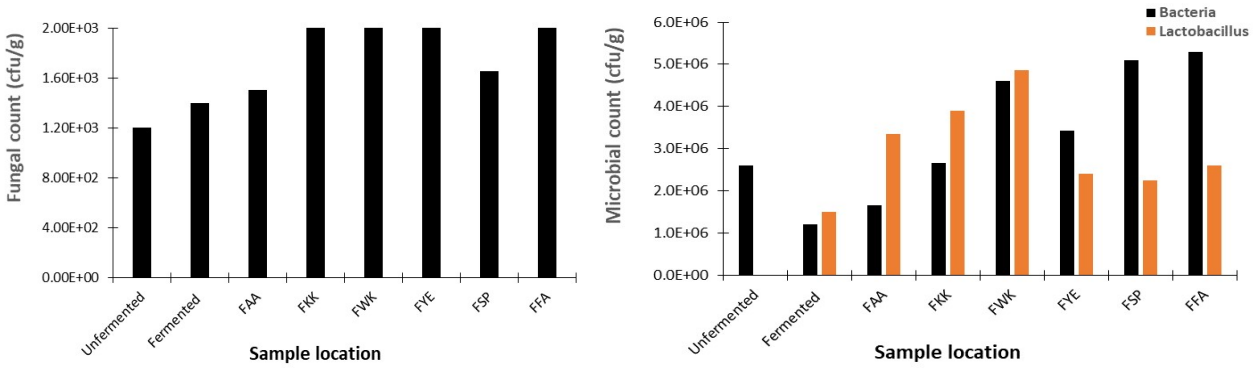

Figure 4: Microbial counts of the*fufu* samples,FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE =Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko; FFA= Oba-Akoko

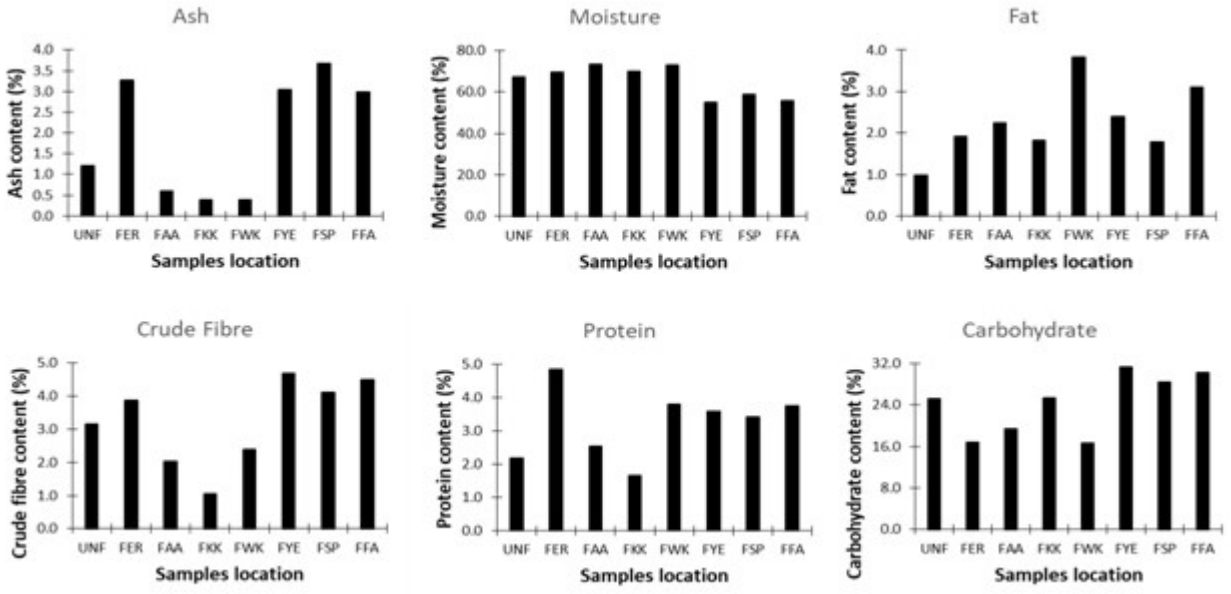

Figure 5: Proximate analysis of the*fufu*samples (%),UNF= Unfermented sample; FER= fermented sample; FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE =Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko; FFA= Oba-Akoko.

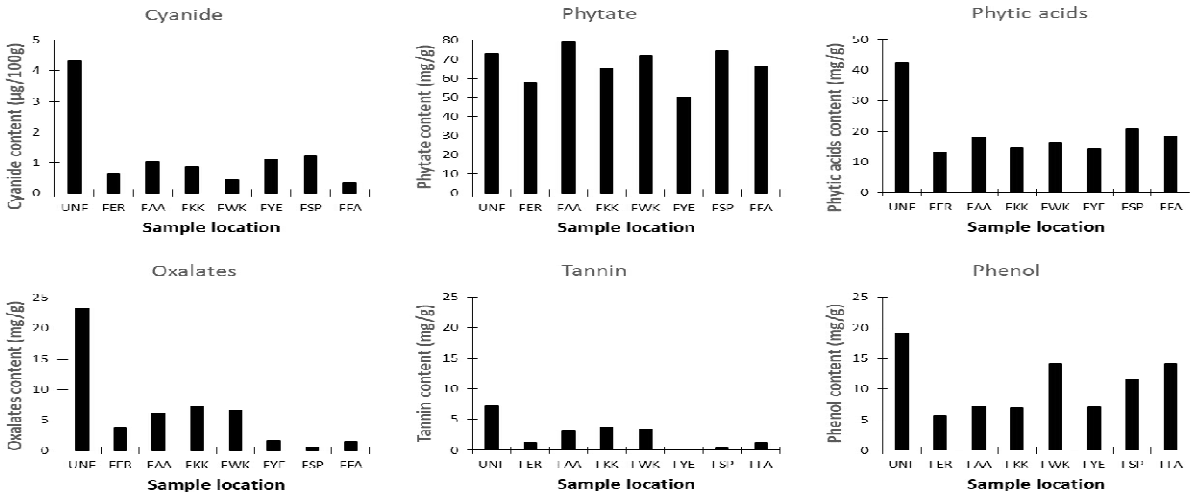

Figure 6: Anti-nutrient analysis of the *fufu* samples,UNF= Unfermented sample; FER= fermented sample; FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE =Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko; FFA= Oba Akoko.

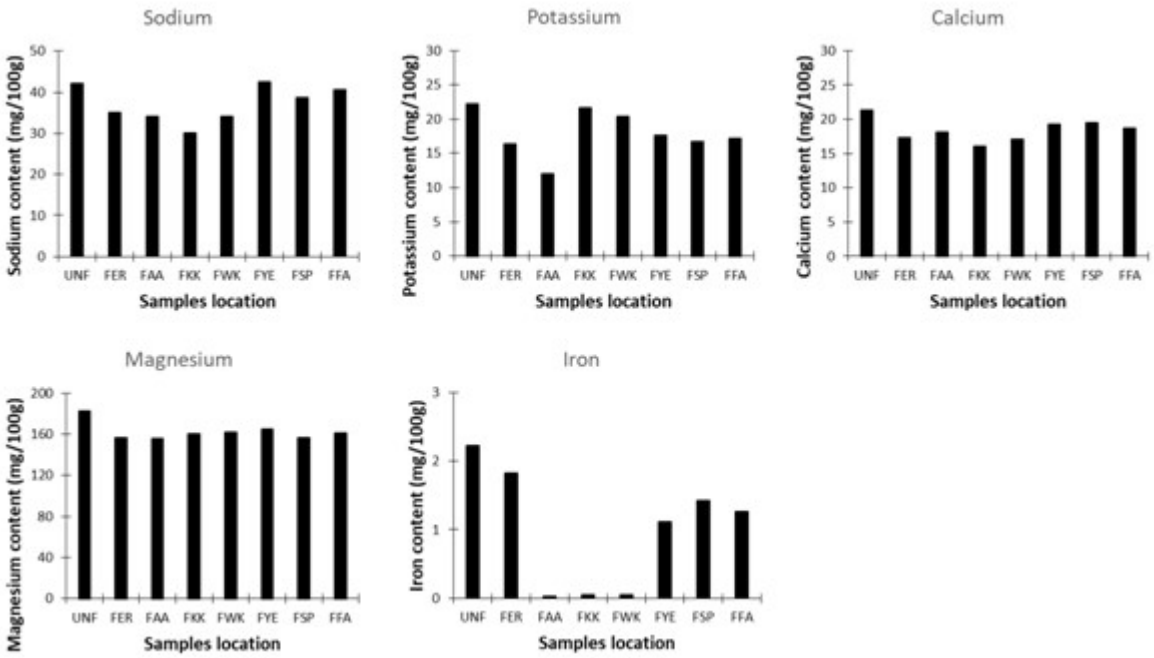

Figure 7: Mineral contents of the *fufu*samples(mg/100g), UNF= Unfermented sample; FER= fermented sample; FAA= Akungba-Akoko; FKK= Ikare-Akoko; FWK= Iwaro-Oka Akoko; FYE =Ayegunle-Akoko; FSP= Supare-Akoko; FFA= Oba-Akoko; FFA= Oba Akoko

IV. DISCUSSION

The most predominant bacteria in all the *fufu* samples were the lactic acid bacteria, with *Lactobacillus plantarum* being the most predominant followed by *L. fermentum*. Many fermented foods particularly the carbohydrate-based foods have been reported to be dominated by lactic acid bacteria. The presence of *B. cereus* and *S. aureus* in some of the *fufu* samples is of great concern because some strains of these bacteria may be pathogenic under certain conditions and have in fact been implicated in food borne intoxication, especially whenfound to be present at 10^5–10^7 cfu/g(Mensah *et al*., 1999; Michelet *et al.,* 2006). *Bacillus cereus* is a common environmental contaminant while *S. aureus* is a normal flora of the human skin. Their presence could be from food handlers, utensils used and the environment in which the food was prepared, handled and stored. Oranusi*et al.*(2007)found that the processing of *fufu* for consumption often involves little or no heat treatment, and if the fairly heat stable toxins from *B. cereus* and *S. aureus* are present in food, it could pose a risk for consumers. *Escherichia coli* which was isolated from samples from three communities in the present study could be from the processing water or feacal contamination and as such not safe for human consumption (Bueno *et al.,* 2004).

Moulds are common environmental contaminants due to their ability to produce spores. This could explain their presence in the *fufu* samples used for this study. While some of the species isolated may be relatively harmless to consumers, some species of *Aspergillus, Fusarium* and *Penicillium* are known to produce deleterious mycotoxins under favourable conditions. Mycotoxins are potent carcinogenic substances which are dangerous to human health (Sweeny and Dobson, 1998; Frisvad*et al.,* 2004;Mazaheri, 2009).

Unfermented *fufu* sample had a pH of 5.1 which is higher than that of the fermented samples. Moreover, the pH value of the *fufu* samples varied from one community to another. For example, the sample purchased from Oba-Akokohad the highest pH value compared with the pH of other samples while the samples purchased from Akungba-Akoko had the lowest pH value. **The total bacterial counts ranged between $1.66x10^6$cfu/g and $5.3x10^6$cfu/g, while for the unfermented sample was $2.6x10^6$cfu/g.The total count for *Lactobacillus* ranged from $2.4x10^6$cfu/g and $4.85x10^6$cfu/g but *Lactobacillus* was not isolated from the unfermented sample, because it had not undergone the fermentation process which is majorly carried out by the lactic acid bacteria (Oyewole and Odunfa, 1988).**

The proximate composition of the samples, the moisture content of all samples ranged from 6.50 % to 73.16%. Samples obtained from Akungba-Akoko, Ikare-Akoko and Iwaro-Oka hadhigh moisture contents while the crude protein, ash and crude fiber contents of all the *fufu* samples were low, and this agreed with the findings of Ngaba*et al.*(1990). However, the carbohydrate contents of all the samples were similar to the findings of Ojo and Akande (2013).

The anti-nutritional factors determined were phytates, phytic acids, oxalates, tannin and phenols. Oxalate has a value which varies in the six samples, phenol has a value that ranged between7.05 mg/100g and 14.09mg/100g.Ojo and

Akande (2013) reported that different processing methods such as cooking, autoclaving and soaking have an influence in reducing the nutritional factors of foods. Lewu *et al*. (2012) found similar results to the mineral composition analysis conducted in this study, where magnesium was the most abundant mineral with values which ranged between 156 to 165mg/100g, and low level of iron.

V. CONCLUSION

The major factors which contribute to the contamination of *fufu* include dirty environment, poor hygiene, and poor quality of water. Therefore, there is need to educate the processors and retailers on the hazards of wet *fufu* processing and on the need to keep proper hygiene to ensure that the food sold to the public are fit for consumption. Intervention strategies that can be employed are washing of cassava tubers with potable water at least twice before soaking, washing of hands and equipment with soap and clean water before and after processing, and packing of wet *fufu* in clean sacks. Contamination and growth of pathogens can also be controlled by defined processing conditions and adherence to proper hygiene during wrapping of the *fufu* with polythene bags. The packaging of *fufu* should also be carefully done in such a way that there would be no exposure to insect vectors which could transfer pathogenic microorganisms to the food.

REFERENCES

[1]. Adegbehingbe, K.T.,Adeleke, B.S. and Adejoro, D. (2017). Microbiologicalassessment, physico-chemical and functional properties of *agidi*produced in Akoko area of Ondo State. *FUOYE Journal of Pure and Applied Science*. 2(1): 275-285.

[2]. Akingbala, J.O., Oguntimehin, B.C. and Abass, A.B. (1991). Effects of processing methods on the quality and acceptability of fufu from low cyanide cassava. *Journal of Science and Food Agriculture.* 57(1): 151-154.

[3]. Alexopoulous, C.J., Mims, C.W. and BlackWell, M.B. (1996). *Introductory Mycology.* John wiley and sons. 1996, ISBN 0-471-52229-5.

[4]. Association of Official Analytical Chemists (2005). *Official Methods of Analysis of the Association of Analytical Chemists International.* 18th ed. AOAC, Gaithersburg, MD.

[5]. Bueno, D.J., Silva, J.O. and Oliver G. (2004). Fungal isolation and enumeration of foods. *Methods inMolecular Biology.* 268: 127-131.

[6]. Cowan, S.T. (1974). Cowan and Steel's manual for the identification of medical bacteria. 2nd edition. Cambridge University Press.

[7]. De Bruijn, G.H. and Fresco, L.O. (1989). Theimportance of cassava in world food production.*NetherlandsJournal of Agricultural Science*. 37: 21-34.

[8]. Frisvad, J.C., Frank, J.M., Houbraken, J.A., Kuijpers, A.F. and Samson, R.A. (2004). New ochratoxin Aproducing species of *Aspergillus* section *Circumdati*. *Studies in Mycology*. 50:23-43.

[9]. Lewu, M.N., Adebola, P.O. and Afolayan, A.J. (2010).Comparative assessment of the nutritional value of commercially available cocoyam and potato tuber in South Africa. *Journal of FoodQuality*. 33:461–476.

[10]. Mazaheri, M. (2009). Determination of aflatoxins in imported rice to Iran. *Food and Chemical Toxicology.* 47: 2064-2066.

[11]. Mensah, P., Owusu-Darko, K., Yeboah-Manu, D., Ablordey, A., Nkrumah, F.K. and Kamiya, H. (1999). The role of streetfood vendors in the transmission ofenteric pathogens *Ghana MedicalJournal*. 33: 19-29.

[12]. Michelet, N.,Granea, P.E. and Mahillon,J. (2006).*Bacillus cereus*enterotoxins, bi- and tri-component cytolysins, and otherhemolysins.In: *TheComparative Sourcebook of Bacterial ProteinToxins*, (Eds. Alouf. J.E. and Popoff, M.R.), Elsievier,Amsterdam, The Netherlands.pp. 779–790.

[13]. Ngaba, P.A and Lee, J.S (1979). Fermentation ofcassava. *Journal of Food Science.*44: 1570 –1573.

[14]. Oluwole, O.B., Olatunji, O.O. and Odunfa, S.A. (2004). A process technology for conversion of dried cassava chips into gari.*Journal of Food Science and Technology*. 22:65–77.

[15]. Ochei, J. and Kolhatkar, A. (2008).*Medical Laboratory Science: Theory and Practice*, McGraw-Hill, New York, NY, USA.

[16]. Oguntunde, A.O. and Orishagbemi, C.O. (1991). Techniques and Technology of cassava tuber processing. *Proceedings of the National workshop on expanding cassava processing options Ijebu- ode Nigeria pp 21-31.*

[17]. Ojo, A. and Akande, E.A. (2013). Quality evaluation of 'gari' produced from cassava and potato tuber mixes. *African Journal of Biotechnology.* 12:4920–4924.

[18]. Okafor, N., Umeh C. and Ibenegbu, C.A. (1998). Amelioration ofgarri, a cassava based fermented food by the inoculation of microorganisms secreting Amylase, Lysine and Linamarase into the cassava mash. *World Journal of Microbiology and Biotechnology.* 14(6): 835-838.

[19]. Okpokiri A.U., Ijeomam, B.C., Alozie, S.O. and Ejiofor MA (1985). Production of improved fufu. *Nigerian Food Journal* 13: 145 – 148.

[20]. Olutiola, P.O, Famurewa, O.F. and Sonntag, H.G. (2000). An introduction to general microbiology. *A Practical Approach Hygiene*. 2: 3-5

[21]. Omafuvbe, B.O., Adigun, A.R., Oguusuyi, J.L. audAsuumo, A.M. (2007). Microbial Diversity in Ready-to-eat Fufu and La fun-Fermented Cassava Products Sold in Ile-Ife, Nigeria. *Research Journal of Microbiology* 2(11): 831-837

[22]. Oranusi, S., Galadima, M., Umoh, V.J. and Nwanze, P.I. (2007). Food safety evaluation in boarding schools in Zaria, Nigeria, using the HACCP system. *Scientific Research and Essay.* 2(10):426-433.

[23]. Oyewole, O.B. andOdunfa, S.A. (1988). Effects of fermentation on carbohydrate,mineral and protein contents of cassava during fufuproduction. *Journal of Food Composition and Analysis*. 2*:* 170-–176.

[24]. Oyewole, O.B. and Ogundele, S.L. (2001). Effect oflength of fermentation on the functionalcharacteristics of fermented cassava fufu. *Journal ofFood Technology.* Africa, 6(2): 38-40.

[25]. Sneath, P.H.A., Mair, N.S., Sharpe, M.E. and Holt, J.G.(1986). *Bergey's Manual of SystemicBacteriology.* Volume 2.

[26]. Sweeney, M. and Dobson, A. (1998). Mycotoxin production by *Aspergillus, Fusarium* and *Penicillium* species. *International Journal of Food Microbiology.* 43(3): 141-158.

[27]. Umeh, S.O. and Odibo, F.J.C. (2013). Production of high protein and low cyanide wet fufu mash using starter cultures. *International Journal of Applied Science and Engineering.* 1: 45-48.