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LEVEL OF MICROBIAL CONTAMINATION OF FRESHLY PREPARED FUFU AND RETAILED FUFU

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ABSTRACT

***Purpose**: Fufu is an acid-fermented products produced through submerged fermentation of cassava root in water for days. The study was set to determine the microbiological quality and the physiochemical composition of freshly prepared fufu (FF) and retailed fufu (RF).*

***Subjects and Methods:** A standard microbiological method was used in enumerating and identify bacteria and fungi species isolated. The total heterotrophic bacteria count ranges from Log_{10} cfu/g 2.14 to 6.18 and 3.56 to 7.06 for retailed fufu and freshly prepared fufu respectively.*

***Results:** Predominant bacteria found in the samples studied and their percentage of distribution were Lactobacillus 17(23%), Escherichia coli and Staphylococcus sp. 14(19%), Bacillus sp. 13(18%), Micrococcus sp. 5(7%), Enterobacter and Corynebacterium were 4(5%) and Proteus has the least 3(4%). Among the fungi were Mucor sp. and Candida sp. were 6(26.1%), Aspergillus niger 4(17.4%), Saccharomyces sp. 5(21.7%), and Rhizopus sp.2(8.7). The proximate analysis revealed an insignificant difference in crude protein ranging from 1.36% in FF to 1.24 in RF, carbohydrates content 82.88% to83.12% in RF and FF, ash content is higher in FF ranging 7.33% to 1.53% RF, while the moisture content is higher in RF with 9.47% and 1.78% in FF. Magnesium ranges from 38.4mg/ml in FF to 35.3mg/ml in RF.*

***Conclusions:** The results obtained showed that the fufu samples that were retailed were more contaminated than the freshly prepared fufu which might pose risk to health. Therefore, there is a need for proper sanitary conditions and best processing and production especially during packing is recommended.*

INTRODUCTION

Cassava (Manihot esculenta) is a tuber plant that serves as an inexpensive source of carbohydrates and other nutrients in the diets of most populations in Africa and Asia, providing energy for an estimated 500 million people (Achi & Akomas, 2006). Nigeria is the world's largest cassava producer, with many cassava producers and processors, mostly smallholders (Forsythe *et al*., 2016). Fufu is a traditional Nigerian fermented food product of southern, western and eastern Nigeria and other parts of West Africa (Rosales *et al*., 2016; Uche, 2016) reports that fufu has higher profitability, better gross margin, profit margin and monetary outlook. According to (Okpokiri *et al*., 2005), at the end of the 20th century, 60% of the total cassava harvested in Nigeria was used to make fufu and only 5% for gari. However, preferences and consumption patterns have been inverted between fufu and gari in recent times, due to fufu's short shelf life and cumbersome processing methods. Fufu is ranked alongside gari as a food native to southern Nigeria (Egwin *et al*., 2013) and it is common in many parts of West Africa (Uyoh *et al*., 2009).

Fufu is an acid fermentation product obtained by soaking cassava roots in water for several days. Fufu is often prepared by rural households and processors whose methods may vary depending on the season and region. Fermentation is an important part of fufu production, a key step in detoxifying tapioca (i.e., breaking down cyanogen glycosides), developing fufu's characteristic aroma and flavor, and helping to preserve it (Flibert *et al*., 2016). During fufu fermentation, lactic acid bacteria, yeasts and other bacteria contribute significantly to starch breakdown, acidification, detoxification and flavor development (Oyewole & Odunfa, 1988). Fufu was first produced by peeling and washing the cassava roots, then cutting them into small pieces. Root pickling/soaking methods vary between states and processors in South-South Nigeria. Soaking, soaking or fermenting the cut roots can be accomplished by continuously soaking the cut roots for a period of 3-5 days for fermentation (Mokemiabeka *et al*., 2011) or by washing and crush the soaked roots after 48 hours of soaking. fermentation, followed by re-soaking of the grated roots (Omodamiro *et al*., 2012) The fermented root or the final grind is sieved and dehydrated to obtain a moist mixture. Sour taste, flavor, appearance and texture are mainly recognized as determinants of the acceptability and quality of fufu (Bamidele *et al*., 2015).

Fufu is produced, sold and consumed in Nigeria and other African countries without any official regulation or certification, which requires microbiological studies in other countries to confirm demonstrate its consumption and quality. Fufu is an acid fermentation product obtained from the submerged fermentation of cassava roots. Foodborne illnesses are caused by injection of food contaminated with pathogenic microorganisms, toxins, chemical and physical agents. Fufu is produced, sold and consumed in Nigeria without any official regulation or certification, requiring microbiological studies in other countries to verify its consumption and quality. The fermentation of cassava roots allows softening for further processing and reduction of potentially toxic cyanogenic glycosides present in the tubers (Achi & Akomas, 2006). Submerged fermentation by traditional methods often produces a foul-smelling mixture due to uncontrolled fermentation and storage techniques (Achi & Akomas, 2006). This type of fermentation, although the simplest way to achieve fufu through cassava fermentation, involves a complex microbial such *Bacillus, Leuconostoc, Klebsiella, Corynebacterium, Geotrichum, Streptococcus, Enterococcus, Aerococcus and Pediococcus species* (Blandino *et al*., 2003). *Yeasts and molds such as Saccharomyces, Candida, Kluyeromyces, Aspergillus, Rhizopus, Mucor, Penicillium and Debaryomyces* were also reported (Omemu *et al*., 2007) The Presence of these organisms could be as a result of post processing contamination from poor handlings such as during mixing, kneading, moulding, hawking and other human activities. The high acidic level that is involved in fermented fufu is sufficient to eliminate most of the microorganisms but post processing contamination may occur which affects the quality of the final product (Odom *et al*., 2012).

The presence of Lactococcus plantarum is an indication that the acid fermentation of fufu is brought about by the lactic acid bacteria (LAB). The presence of Staphylococcus aureus could be due to contamination from the skin, mouth, or nose of the handlers or hawkers since it is a member of the normal flora of the skin and the though the percentage of the isolate is high, this does not portray a serious concern since the temperature of most of the fufu is raised before final consumption. *Bacillus cereus*, an opportunistic pathogen of humans, is a frequent inhabitant of soil, leaf surfaces and wrapping materials. Its presence in the fufu may results from the soil and materials used in wrapping, covering the fermentation tank or drum and packaging (Odom *et al*., 2012). *Aspergillus spp* in the food can leads to food poisoning, since many of these fungi are toxin- producing organisms. The presence of *Escherichia coli* in the food indicates that such fufu has been contaminated with faecal materials and such food might not be safe for human consumption.

The odour from fufu discourages some people from consuming it, however some people still delight in consuming it. Reduction of the odour in fufu by enhanced technology (optimization) will make it to be more liked and generally acceptable to a wider population. This research work was aimed at determining the microorganisms associated with retailed and freshly prepared fufu as well as the physicochemical analysis of fufu sold in Port Harcourt.

METHODOLOGY

Sample Collection and Procedure

A total of 32 samples, ten (Cheesbrough, 2006) (freshly prepared) fufu and ten (Cheesbrough, 2006) retailed fufu were randomly procured locally using a simple random sampling technique were bought from vendors at Woji, in Port Harcourt Rivers State, Nigeria and were cultured in a sequence of day 1, day 3, day 5, day 7, day 9, day 11, day 13, day 15 in duplicate.

Plate1; Samples of fufu

Samples Preparation

25g of samples into 225ml of peptone water then homogenized in a stomacher to get the stock solution. Tenfold serial dilution was done from 10^1 to 10^7 then 0.1ml were inoculated into the Petri dishes containing Potato dextrose agar (PDA), MacConkey agar, Mannitol salt agar, Plate count agar (PCA) and spread, this follows incubation.

Microbial Analysis: Enumeration of the total Bacterial Count, Total Staphylococcus Count, and total Coliform Count.

Counts from the incubated agar plates were enumerated after 24hours for bacteria and 72 hours for the fungal count. Determination of Colony Forming Unit (CFU/g**)** The cfu/g was determined using Cfu/g = Number of colonies x dilution factor / Volume of the culture plate.

Isolates Morphological Identification of bacteria

Bacterial isolates were characterized and identified using cultural, morphological and microscopic examinations. The macroscopic examination of the colonies was differentiated based on size, color, pigmentation, elevation surface texture and margin. and biochemical tests such as Gram staining, Catalase, Coagulase, Methyl-red, Oxidase, Voges-Proskauer and sugar fermentation test were employed to differentiate the bacterial isolates according to the standard microbiological methods as described by (Cheesbrough, 2006).

Fungal Identification

Identification of all fungal isolates was also carried out using standard methods based on macroscopic and microscopic features as described by Lacto-phenol (Cotton blue test). On a clean slide, a drop of methanol was placed and a portion of fungi growth was cut with the aid of a surgical blade and tested in the methanol. A drop of lacto-phenol cotton blue was added. A cover slip was placed on it gently and observed under the microscope with X40 objectives.

Proximate Analysis The moisture, ash, fats and oil, fiber, protein and carbohydrate content. was done as described by (Obinna *et al*., 2012).

Mineral determination: The sodium, calcium, potassium, magnesium and zinc contents of all fufu samples were determined as described by (AOAC, 1990).

Statistical Analysis Microbiological data from several fruit samples were obtained and entered into a Microsoft Excel spreadsheet before being evaluated with a one-way ANOVA using statistical software from the Statistical Package for Social Sciences (SPSS) version 16. Statistical significance was defined as a 95% confidence interval with a P value of 0.05 or lower

RESULTS AND DISCUSSION

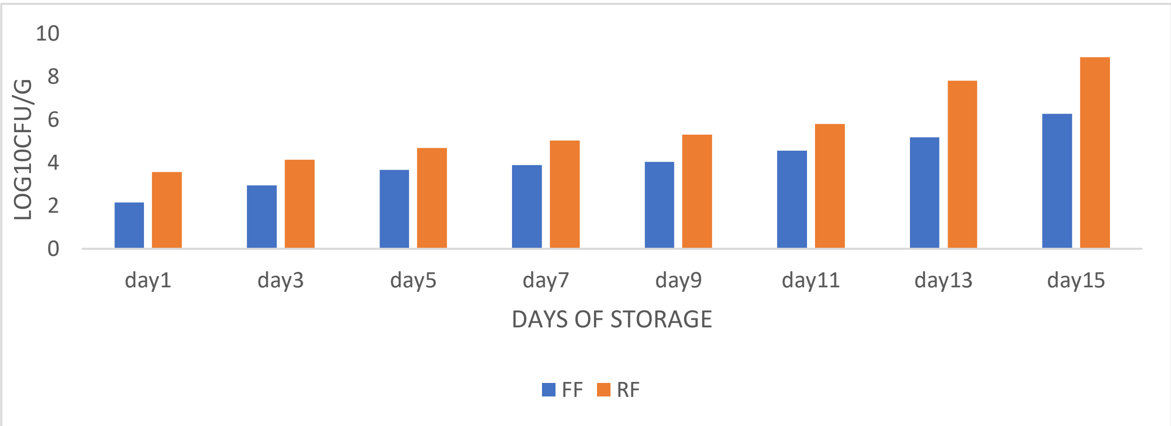

Figure 1: Total heterotrophic bacteria count obtained from freshly prepared fufu (FF) and retailed fufu

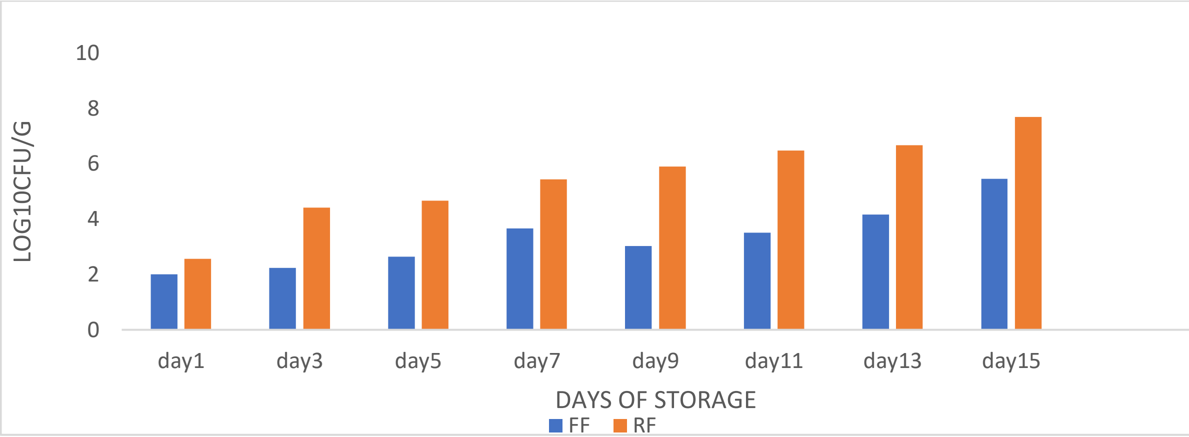

Figure 2 Total Staphylococcus count obtained from freshly prepared fufu (FF) and retailed fufu (RF)

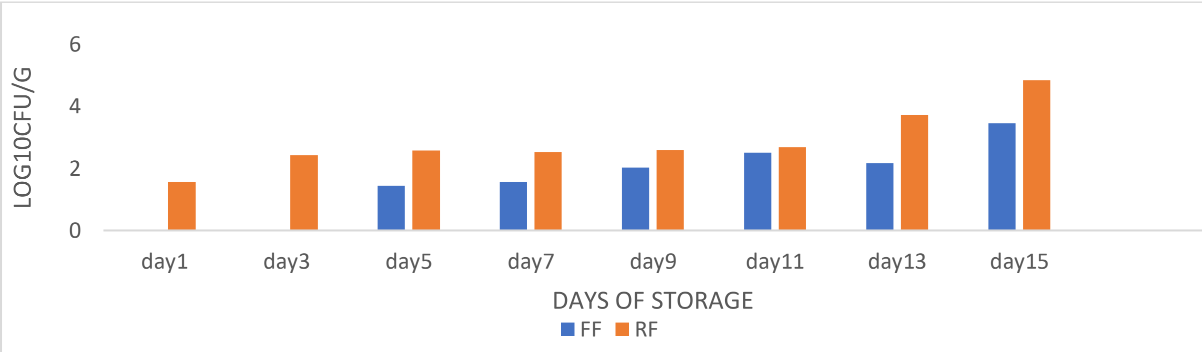

Figure 3. Total coliform count obtained from freshly prepared fufu (FF) and retailed fufu (RF)

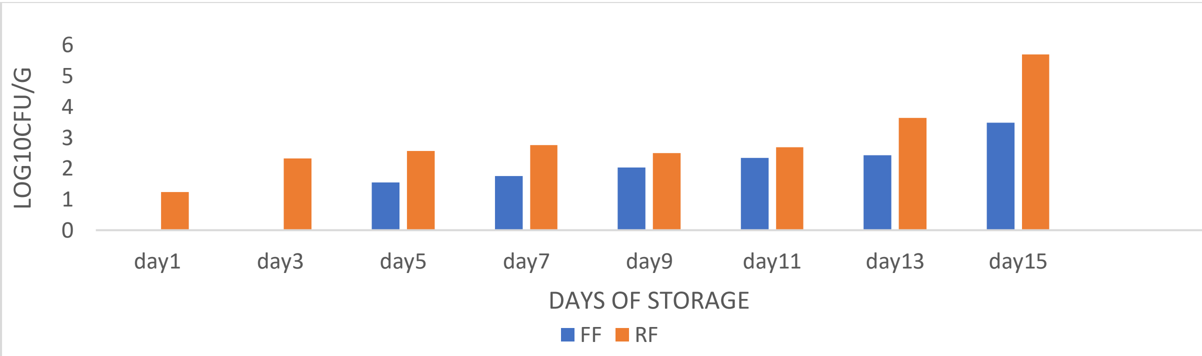

Figure 4 Total fungal count obtained from freshly prepared fufu (FF) and retailed fufu (RF)

Table 1: Percentage Occurrences of the different bacteria isolated from freshly prepared and retailed fufu samples.

Organisms	No. of Occurrence	Frequency (%)
Bacillus sp.	13	18
Escherichia coli	14	19
Staphylococcus sp.	14	19
Corynebacterium sp.	4	5
Proteus	3	4
Micrococcus	5	7
Lactobacillus	17	23
Enterobacter sp.	4	5
Total	74	100

Table 2: Percentage Occurrences of the different fungi isolated from freshly prepared and retailed fufu samples.

Organism	No. of Occurrence	Frequency (%)
Candida sp.	6	26.1
Saccharomyces sp	5	21.7
Mucor	6	26.1
Aspergillus sp	4	17.4
Rhizopus	2	8.7
Total	23	100

Figure 5; shows the proximate composition of freshly prepared fufu were 5.78% moisture content. crude protein (1.24%), crude lipid (1.14%), crude fiber (3.75%), Ash (1.53%) and carbohydrate (82.88%) while retailed fufu has (7.33%) Ash, (1.36%) protein, (1.28%) lipid (5.13%) fiber, (83.88%) carbohydrate

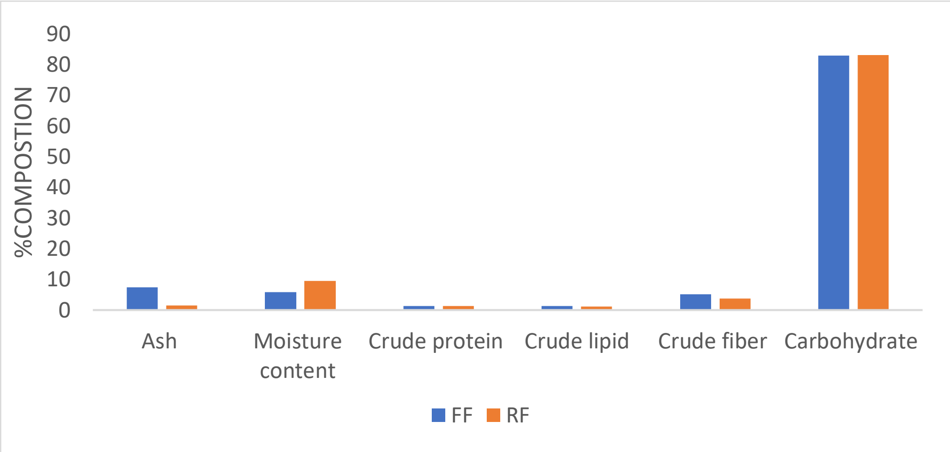

Figure 5: Mean proximate Composition of Retailed and Freshly Prepared Fufu

Figure 6; shows the mineral composition of freshly prepared and retailed fufu, 351mg/ml of potassium, 384.4 mg/ml of sodium, 56.2 mg/ml calcium, 32 mg/ml of iron, 5.1 mg/ml of zinc, 38.4 mg/ml magnesium were higher compared to the mineral composition of retailed fufu having 344 mg/ml of potassium, 355.2 of sodium, 544mg/ml of calcium, 30.5 mg/ml, 4.6 mg/ml of zinc and 35.3 mg/ml magnesium**.**

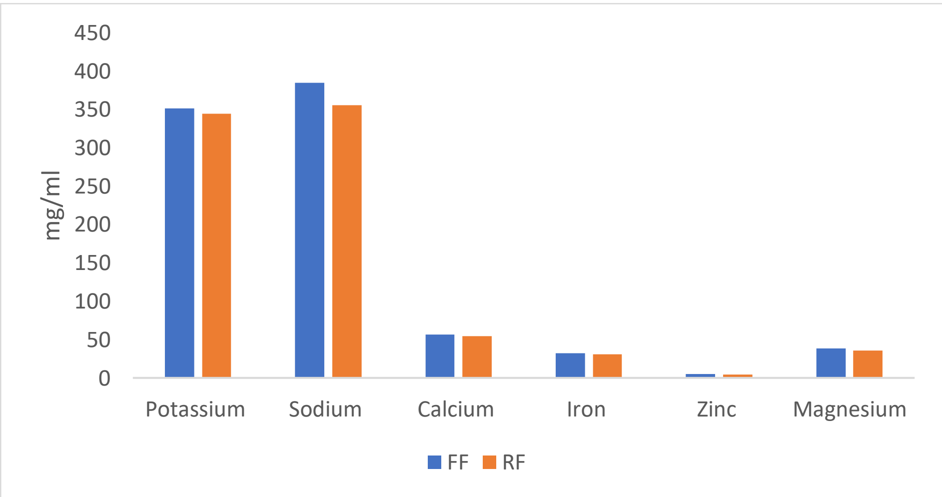

Figure 6.: Mineral composition of freshly prepared fufu and retailed fufu

Microbial Contamination of Freshly Prepared Fufu and Retailed Fufu.

An acid-fermented product called "fufu" is made by fermenting cassava roots in water for several days. Cassava root can be softened for further processing and its potentially hazardous cyanogenic glucoside can be reduced through fermentation. (Achi & Akomas, 2006). These procedures require intricate microbiological mechanisms (Obadina *et al.*, 2007). The total heterotrophic bacteria count as shown in Fig 1- for the duration of storage indicated that retailed fufu ranged from Log_{10}cfu/g 3.56 to 8.89, while freshly prepared fufu ranged from 2.14 to 6.26. There was a mediated increase in bacteria count with an increase in storage time. Significantly higher counts were recorded in retailed fufu compared to freshly prepared fufu ($p<0.05$). International commission of microbiological specification for food (ICMSF, 2009) reported that plate count within the range of $5.0x\ 10^5$ is considered acceptable in food. Most of the fufu samples from Day 7 exceeded the stated microbial limits and were unfit for consumption.

This could be ascribed to the processor's or the vendors' methods of storage or production procedures. These organisms may be present as a result of improper handling during mixing, kneading, molding, hawking, and other human actions, as well as post-processing contamination. Although most microorganisms can be removed by the fermented fufu's high acidity level, post-processing contamination can occur and lower the end product's quality (Odom *et al*., 2012).

Since the fermentation used to produce the fufu product was natural fermentation, which involves a complex microbial population during fermentation, the microbes recovered from the fufu samples may be the organisms involved in the fermentation. Despite the possibility that this is the case, it is indisputable that some of these microorganisms may have been introduced by humans, packaging materials, storage temperatures, pH levels, or even the immediate location where the processed cassava was stored (Odom *et al.*, 2012). Total staphylococcus counts as shown in Fig 2 for the duration of storage indicated that freshly prepared fufu ranged from Log_{10}cfu/g 2.01 to 5.98, while retailed fufu ranged from 2.56 to 7.70. A major public health concern is the presence of the harmful bacterium Staphylococcus aureus. Even though the meal may look appetizing to the eye, Staphylococcus aureus produces various heat-stable extracellular chemicals known as enterotoxins that make the food dangerous. These enzymes aid in the Staphylococcal invasiveness of the immune system (Prescott *et al*., 2005). Depending on the amount of inoculum consumed and the individual’s susceptibility to the toxin, the intensity of the symptoms may change. Vomiting, nausea, diarrhea, and abdominal pain are a few signs of staphylococcal food poisoning (Amusan *et al.*, 2010). The environment in which food is prepared, handled, and kept as well as the person handling it can all harbor Staphylococcus bacteria. According to (Mbayei & Iroegbu, 2010), air pollution and other environmental factors are potentially potential sources of Staphylococcus. The appearance of Staphylococcus aureus is largely due to human interface. This reflects unclean practices during production and sale, as the organism is usually found on the skin and nose, may be due to contamination from the mouth or nose. The presence of Staphylococcus aureus can be due to contamination from the skin, mouth or nose of the handlers, as it is a member of the normal skin flora. Total coliform counts as shown in Fig 3 for the duration of storage indicated that retailed fufu ranged from Log_{10}cfu/g 1.56 to 3.46, while freshly prepared fufu ranged from1.45 to 4.85. In general, most strains of E. coli are harmless and are part of the normal gut flora, although some serotypes cause serious food poisoning in humans. *E. coli* can be found in the urinary tract, which is its natural habitat. The main route of *E. coli* of the fufu samples in this study may have come from the water used to wash the mortar and pestle before or from the water used to mold or beat the fufu, an unhygienic environment may also contribute and the storage for a long time by the seller if not sold in time. The presence of Escherichia coli in the food indicates that such fufu has been contaminated with faecal materials and such food might not be safe for human consumption. The acceptability of the fufu mill depend on the types of bacteria isolated from the fufu. The isolated bacteria include *Lactobacillus sp* 17(23%), *Escherichia coli* and *Staphylococcus sp* 14 (19%), *Bacillus sp* 13(18%), *Micrococcus sp* 5 (7%) *Enterobacter* and *Corynebacterium sp*. 4(5%,) and *Proteus* 3 (4%). The most predominant bacteria were the *Lactobacillus* 17(23%) as shown in Table1. The result is in agreement with the findings reported by (Adegbehingbe *et al*., 2019). Fermented foods especially carbohydrates-based foods have been reported to be dominated by lactic acid bacteria. Total fungi count as shown in Fig 4 for the duration of storage indicated that retailed fufu ranged from Log_{10}cfu/g 1.23 to 4.75, while freshly prepared fufu ranged from1.5 to 3.49. *Aspergillus spp* in the food may lead to food poisoning, since many of these fungi are toxin producing organisms, ubiquitous in the environment and originated from the market display areas. *Aspergillus spp* in the food can leads to food poisoning, since many of these fungi are toxin-producing organisms.

The isolated fungi molecules *Mucor sp* and *Candida sp* were 6 (26.1%), *Aspergillus niger* 4(17.4%), *Saccharomyces sp* 5(21.7%) and *Rhizopus 2(8.7%)* as shown Table 2 The Mucor sp and Aspergillus niger sp is the most predominant fungi isolate from freshly prepared fufu and retailed fufu. This could be due to the ability to withstand adverse conditions due to the presence of spores. The result agreed with the findings reported by Adegbehingbe et al, (2019).

Physiochemical Composition of Freshly Prepared Fufu and Retailed Fufu

Mean Proximate composition of the samples as shown in fig 5 the moisture content of freshly prepared fufu and retailed fufu were 5.78% and 9.47% respectively. The variation in moisture content observed from the different fufu samples in this study may be due to their ability to absorb moisture under storage conditions, which subsequently promotes the colonization of spoilage fungi. damage and increase their deteriorative capabilities. Moisture absorbed by fufu samples enhanced the biodegradability of Moisture absorbed by fufu samples enhanced the biodegradability of these microorganisms (Jonathan et al., 2016). Traditionally, fufu was sold as a wet paste, making it highly perishable and reducing its shelf life (Tomlins et al., 2007).

Crude protein of freshly prepared fufu and retailed fufu were 1.24% and 1.28% respectively. The low protein content of fufu has been confirmed in the nutritional composition of cassava roots, where carbohydrates are the major nutrient, 80% of which is starch (Purseglove, 2009). However, the protein content in this study was lower than the results of (Etudaiye et al., 2012), which he ranged from 2.10% to 2.46% and similar to the results of (Amadi et al., 2019). The low protein content of fufu is not a serious problem, as fufu is usually consumed with a variety of protein sources, both animal and vegetable, from a variety of soups (Montagnac & Davis, 2009). Many attempts to improve cassava protein through biofortification and post-harvest treatments were reported, with some success reported (Ayetigbo *et al*., 2018). Also reported that protein content was significantly higher in white-meat cassava varieties than in yellow-meat varieties. Crude lipid of 1.14% and 1.36% for freshly prepared fufu and retailed fufu respectively. Fats are vital to the structure and biological functions of cells which are used as an alternative energy source. Also, (Ayetigbo *et al*., 2018) reported fat content of 0.2 to 3.66% for white flesh cassava and 0.29 to 3.2% for the yellow- fleshed cassava in their study. This was different with the results obtained in this study. Crude fiber of 3.75 % and 5.13% was obtained for freshly prepared fufu and retailed fufu respectively. The fiber content of cassava fufu varies with cassava species and age at harvest (Montagnac & Davis, 2009). As reported, relatively high fiber content between 0.62% and 4.92%, found in yellow-fleshed species (Ukenye *et al*., 2013), slightly lower than in this current study. Crude fiber, which represents the portion of the diet that is not used by the body, is composed primarily of cellulose and lignin and is known to facilitate bowel movements (Eleazu *et al*., 2011). Crude fiber is mainly composed of cellulose and lignin, and some minerals.

Ash content of 7.33% and 1.53% was obtained for retailed fufu and freshly prepared fufu respectively. Ash is an important component of cassava and indicates cassava's rich mineral and non-volatile content (Montagnac & Davis, 2009). The study reported that total non-combustible matter (ash) in fufu was higher in commercial fufu than in freshly made fufu. Processing of cassava has been reported to significantly reduce root ash, with similar trends for minerals (Ogden & Bhatt, 2006). Carbohydrate content of 82.88% and 83.88% was obtained for freshly prepared fufu and retailed fufu respectively. However, differences in genotype and age can also lead to differences in carbohydrate content between different species. The results of this study are in agreement with (Ukenye *et al*., 2013), who also reported high carbohydrate content also (Okolie *et al*., 2012) reported a similar carbohydrate content of 85.8%. The decrease in carbohydrate content in the fufu samples may be due to the preliminary amount of soluble carbohydrates lost during the processing of cassava roots (Chiedozie *et al*., 2018). The mineral composition of freshly prepared and retailed fufu, 351mg/ml of potassium, 384.4 mg/ml of sodium, 56.2 mg/ml calcium, 32 mg/ml of iron, 5.1 mg/ml of zinc, 38.4 mg/ml magnesium were higher compared to the mineral composition of retailed fufu having 344 mg/ml of potassium, 355.2 of sodium, 544mg/ml of calcium, 30.5 mg/ml, 4.6 mg/ml of zinc and 35.3 mg/ml magnesium. The amount of minerals in the analyzed fufu showed great variation. Such variation can be attributed to many factors such as processing, storage and agronomic conditions. Sodium is essential for blood pressure control. It is an electrolyte that controls the amount of extracellular fluid in the body needed for hydration. In addition, sodium stimulates muscles and nerves. The sodium content in most of the dishes analyzed can be considered relatively low compared to the RDA of 1.5 g/day. Except for the excessive use of dietary salt and sodium-containing compounds such as monosodium glutamate (MSG) used in cooking, the consumption of these dishes cannot be a cause for concern or a risk factor. have cardiovascular disease (CVD). Excess sodium intake is associated with high blood pressure and hardening of the artery walls, thus being a risk factor for CVD (Ha, 2014). Calcium is the most abundant mineral in the body and its functions include the regulation of muscle contractions, including heart rate, blood clotting and the formation of strong bones and teeth (WHO, 2004a; WHO, 2004b). Iron is the most common micronutrient deficiency in the world. Women of childbearing age are the group most at risk from menstrual blood loss, pregnancy and lactation. Iron makes it possible to participate in redox reactions with some metalloproteins such as hemoglobin, myoglobin, cytochrome enzymes and many oxidases and oxygenases. It is needed for many proteins and enzymes, including hemoglobin to prevent anemia. Anemia has been shown to be associated with maternal mortality and preterm birth (Carriaga *et al*., 1991). Low potassium levels are associated with an increased risk of high blood pressure, heart disease, stroke, arthritis, cancer, digestive disorders, and infertility. For people with low potassium, a fortified diet — or potassium supplements to prevent or treat some of these conditions may be recommended. Potassium was below the recommended level in the food samples tested. There is strong evidence that reducing dietary sodium and increasing potassium intake reduces blood pressure the incidence of hypertension, and the morbidity and mortality from cardiovascular disease (Carriaga *et al*., 1991). The dietary intake of zinc was adequate compared with the 11 mg/day recommendation in the foods studied. Zinc is a component of more than 100 enzymes, including DNA polymerase, RNA polymerase and RNA synthetase transfer. Zinc deficiency has the most profound effects on rapidly proliferating and retarded tissues in mildly deficient children. More severe deficiency leads to growth retardation, teratogenicity, hypogonadism and infertility, non-healing wounds, diarrhea, dermatitis on extremities and around Orifices, glossitis, alopecia, loss of ability dark adaptation and cellular immunodeficiency (Whelton, 2014; Ringsted *et al*., 1990). Dietary zinc supplementation reduced diarrhea in infants (Sazawal *et al*., 1996). While zinc showed an inverse relationship with dental caries. A component of several metal enzymes, most manganese is found in mitochondria where it is a component of manganese superoxide dismutase. Micronutrients are a variety of food ingredients essential for the maintenance of health. The physiological role of micronutrients is as diverse as their composition; some micronutrients are used in enzymes as coenzymes or pseudo groups, others as biochemical substrates or hormones; in some cases, the functions are not well defined. Under normal circumstances, the average daily intake for each micronutrient required to maintain normal physiological functions is measured in milligrams or less.

CONCLUSION

Fufu is an important cassava production in Nigeria. The results obtained showed that retailed fufu was contaminated with several microorganism and had more microbial load which might be attributed to the longer stay of retailed fufu. Hawking and storage of fufu at ambient temperature for more than 5 days predisposes the fufu to microbial growth which might be detrimental to health. The longer the time of storage, the higher the possibility of being contaminated and the more the microbial load. The microbial contamination might be attributed to improper handling at processing, packaging at market place could to post contamination.

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