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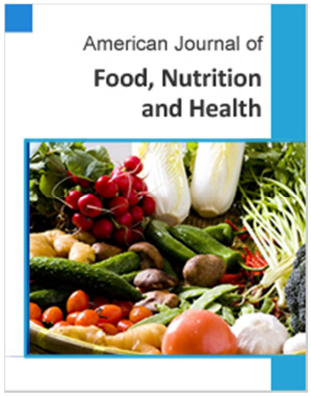

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Microbiological Quality of Commercially Ready-to-Eat *Fufu* Sold in Benin City, Nigeria

Inetianbor Jonathan Ewanfo[1, *], Ikenebomeh Marcel James[2], Udochukwu Ugueri[3]

[1]Department of Microbiology, Faculty of Pure and Applied Sciences, Federal University Wukari, Wukari, Nigeria
[2]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria
[3]Department of Biosciences, College of Natural and Applied Sciences, Salem University, Lokoja, Nigeria

Email address
inetianbor@fuwukari.edu.ng (I. J. Ewanfo)
[*]Corresponding author

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Abstract
Fufu, an acid-fermented cassava product produced through submerged fermentation of peeled cassava roots in water is traditionally produced and consumed in Nigeria and other West African countries and this necessitate microbiological study to ascertain its consumption safety and quality. Fifty (50) samples of ready-to-eat *fufu* were purchased from five different markets (Uselu, Oba, Aduwawa, New Benin and Santana) and they were immediately taken to the laboratory for analyses employing standard microbiological and biochemical assays. The mean colony forming unit per gram (cfu/g) of the bacterial isolates ranged from $9.2 \pm 8.4x10^7$ (Aduwawa market) to $10.1 \pm 8.6x10^7$ (Uselu market) and the mean colony forming unit per gram (cfu/g) of the fungal isolates ranged from $5.1 \pm 4.4x10^7$ (Santana market) to $5.6 \pm 4.9x10^7$ (Oba market). Bacteria isolated were *Enterobacter aerogenes*, *Bacillus cereus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli* and *Lactobacillus plantarum* and the fungi isolates were *Penicillium candidum*, *Rhizopus oryzae*, *Fusarium oxysporum*, *Aspergillus niger*, *Saccharomyces cerevisiae* and *Candida albicans*. The presence, number and the kind of microorganisms in the *fufu* from the different markets in Benin City could be as a result of post processing contamination because the production process does not normally favour the proliferation of these microbes either from the handling, mixing, kneading, moulding and dispensing of the *fufu*. Good personal hygiene and sanitation from the handlers of *fufu*, heating of the *fufu* prior to consumption and close monitoring of the production processes by relevant agencies is highly recommended.

1. Introduction

Fufu is an acid-fermented cassava product produced through submerged fermentation of peeled cassava roots in water and consumed in Nigeria, West African countries and other parts of the world. Cassava (*Manihot esculenta* Crantz) is the staple root crop of over 800 million people in the humid tropics and ranks sixth in terms of overall global crop production (Nassar *et al*., 2007; Han *et al*., 2001). It is a perennial woody shrub with an edible root, which grows in tropical and sub-tropical

areas of the world (Burrell, 2003). Cassava is one of the most important food crops in the tropics that serve as a food security and income generation crop for many millions of people in the developing world (Scott *et al*., 2002). It is one of the most important food staples in the tropics, where it is the fourth most important source of energy (Hillocks *et al*., 2002). Worldwide, it is the sixth most important source of calories in the human diet (El-Sharkawy, 2004) and the third most important in the tropics after rice and maize (Prakash, 2006). Cassava is grown widely in Nigeria and many other regions (countries) of the tropics, where it serves as one of the basic food source for about 200-300 million people (FAO, 2010). The main cassava food products of considerable domestic importance in Nigeria are garri in the south, lafun in the west and *fufu* in the east (Olopade *et al*., 2014). The principal parts of the mature cassava plant expressed as a percentage of the whole plant are 6% leaves; 44% stems and 50% storage roots. The roots and leaves of the cassava plant are the two nutritionally valuable parts, which serve as food. The cassava root is composed of 60-65% moisture, 20-31% carbohydrate, 0.2-0.6% ether extracts, 1-2% crude protein and a comparatively low content of vitamins and minerals. The root carbohydrate is made up of 64-72% starch. However, the roots are rich in calcium and vitamin C and contain a nutritionally significant quantity of thiamine, riboflavin and nicotinic acid that are lost during processing (Nweke *et al*., 2002).

During fermentation of *fufu*, lactic acid bacteria, yeast and other bacteria contribute significantly to starch breakdown, acidification, detoxification and flavour development (Oyewole 1991). Among the fermented products of cassava, *fufu* is one of the favourite consumed in many parts of West Africa countries (Uyoh *et al*., 2009). This is produced by steeping in water peeled cassava cubes for 24-72 hours. The fermented cassava is sieved to remove the fibers and allow to sediment. After sedimentation, the water is decanted and the sediment is dried, milled and the *fufu* flour is obtained (Sanni *et al*., 2007; Tomlins *et al*., 2007).

Fufu 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. There is little or no microbiological study and analysis of commercial ready-to-eat *fufu* sold in Benin City, South-South Nigeria unlike other part of the country like the South-East (Imo and Abia state) where Odom and his colleagues had carried out research work on the Biochemical qualities of cassava *fufu* sold in Imo and Abia States of Nigeria (Odom *et al*., 2012) as well as the South-West (Ogun state) where Olopade and his colleague work on the Microbiological quality of fermented *garri*, a staple food from cassava (Olopade *et al*., 2014). This research work was aimed at determining the microbial load of *fufu* as well as characterizing, isolating and identifying both bacterial and fungal associated with the *fufu* sold in Benin City.

2. Material and Methods

2.1. Study Area

This research work was carried out in Benin City, Edo state, Nigeria. Benin City is located on latitude 6.34°N, longitude 5.63°E and 80 meters elevation above the sea level.

2.2. Collection of Sample

Fifty (50) samples (Ten from each market) of ready to eat *fufu* were purchased from five markets (Uselu, Oba, Aduwawa, New Benin and Santana) in Benin City. They were placed into sterile polythene bags and were taken to the Laboratory within an hour for analysis

2.3. Serial Dilution, Culturing and Biochemical Testing

One grams from each of the *fufu* sample from a vendor in Uselu market was added onto a beaker containing 10ml of distilled water in a ratio of 1:10 and was minced until a paste of the *fufu* was formed. This serves as the stock solution and it was labeled as such. A ten-fold serial dilution was carried out as previously described (Inetianbor *et al*., 2014). The same procedure was repeated for the other *fufu* sample obtained from other vendors in Uselu market and also with the samples from the other markets (Oba, Aduwawa, New Benin and Santana). Bacteria were grown in nutrient agar (NA) and where grown at 37°C for 24H and for the lactic acid bacteria, Deman Rogosa Sharpe (MRS) agar was used and were grown at room temperature for 24H. Pure cultures of the different isolates were obtained and stored in a nutrient broth slant as described (Cheese, 2006). For fungi isolates, the inocula were grown in potato dextrose agar (PDA) for 96H at room temperature (Udochukwu *et al*., 2016). Cultural and morphological characterization of bacterial and fungal isolates were determined according to (Holt *et al*., 1994).

2.4. Biochemical Test

Test such as catalase, oxidase, coagulase, urease, indole production, citrate utilization. Methyl red (MR) and Voges Proskeuer (VP) as well as substrate utilization like glucose, lactose and sucrose were carried as previously described (Cheesbrough, 2000).

3. Results

In order to determine the bacterial load of the ready-to-eat *fufu* in the sampled markets in Benin City, Nigeria, serial dilution of the various samples was carried out and the bacterial load was determined at a dilution unit of 10^{-5} and the result is as showed in figure 1. The highest bacterial count (load) was found in Satana market and the least in Oba market. Figure 2 shows the fungal load determined at 10^{-5} dilution factor. Uselu and Satana markets have the highest

fungal count while oba market had the least count. The percentage of the bacterial and fungal isolated from the various markets are shown in figure 3. *Escherichia coli* has the highest number of bacterial isolates while *Pseudomonas aeroginosa* has the least (figure 3A). The highest fungal isolate was *Saccharomyces cerevisiae* while the least was *Rhizopus oryzae* (figure 3B). In order to determine and isolate the bacteria from the samples, there was need to carryout biochemical assay so as to know the substrate the isolates were able to mineralized. Table 1 shows the various biochemical tests carried out according to the utilization of the substrate and or the production of gas or acids.

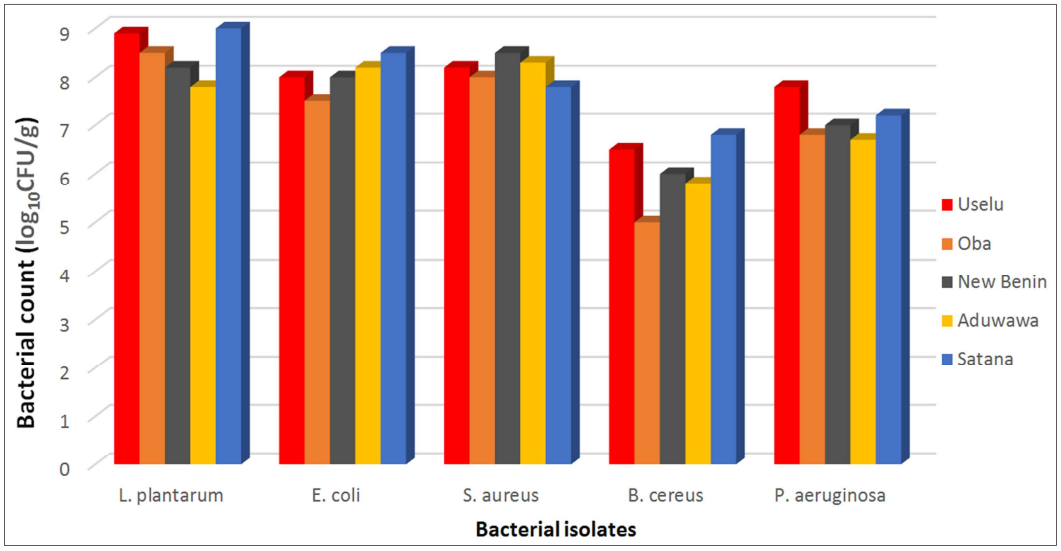

Figure 1. *Bar chart of the bacterial count of the isolates from the various sample markets in Benin City. The highest bacterial count (Lactococcus plantarum) was found at Satana market and the least count, Bacillus cereus was found at Oba market.*

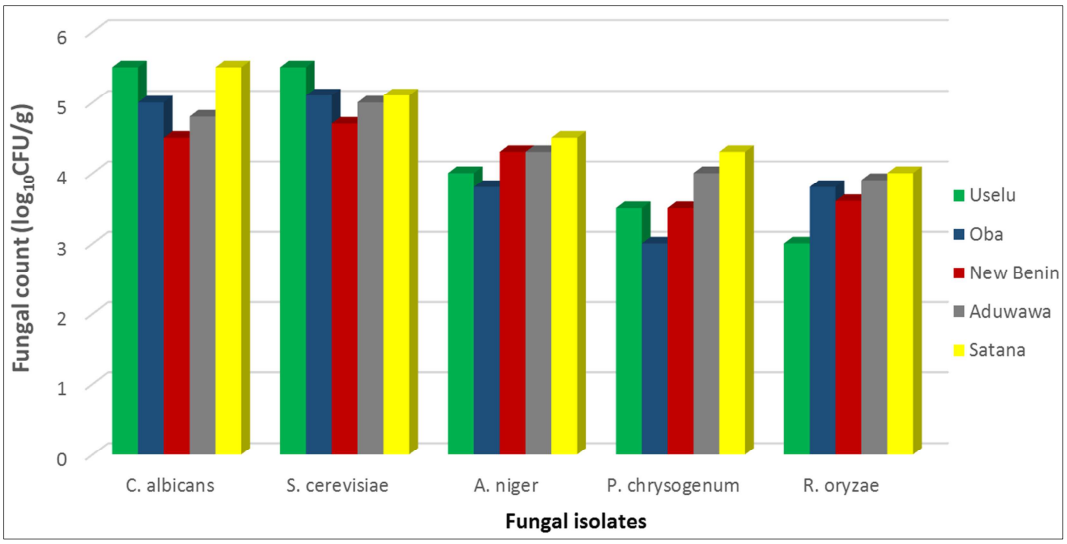

Figure 2. *Bar chart of the fungal count of the isolates from the various sample markets in Benin City. The highest fungal counts (Candida albicans and Saccharomyces cerevisiae) were found at Uselu and Satana markets and the least counts (Penicillium chrysogenum and Rhizopus oryzae) were found at Oba and Uselu markets.*

Table 1. *Biochemical characterization of Bacteria Isolates associated with fufu sample.*

Bacteria isolates	OXI	CAT	COA	URE	MOT	IND	CIT	MR	VP	GLU	LAC	SUC
Staphylococcus aureus	-	+	+	-	-	-	+	+	+	AG	A	A
Escherichia coli	-	+	-	-	+	+	+	-	+	AG	A	A
Bacillus cereus	-	+	-	-	-	-	+	+	-	A	A	A
Enterobacter aerogene	-	+	-	-	+	-	+	-	+	A	A	-
Pseudomonas aeruginosa	+	+	-	-	-	-	+	-	-	AG	-	A
Lactococcus plantarum	ND	-	ND	ND	-	ND	ND	+	-	A	A	A

Key: CAT = catalase, OXI = oxidase, COA = coagulase, URE = urease, MOT = Motility, IND = indole production, CIT = citrate utilization. MR = Methyl red, VP = Voges Proskeuer, GLU = Glucose, LAC = Lactose, SUC = Sucrose, ND = Not Determined, AG = Acid and gas production, A = Acid production, + = Positive reaction/response and - = Negative/ no reaction.

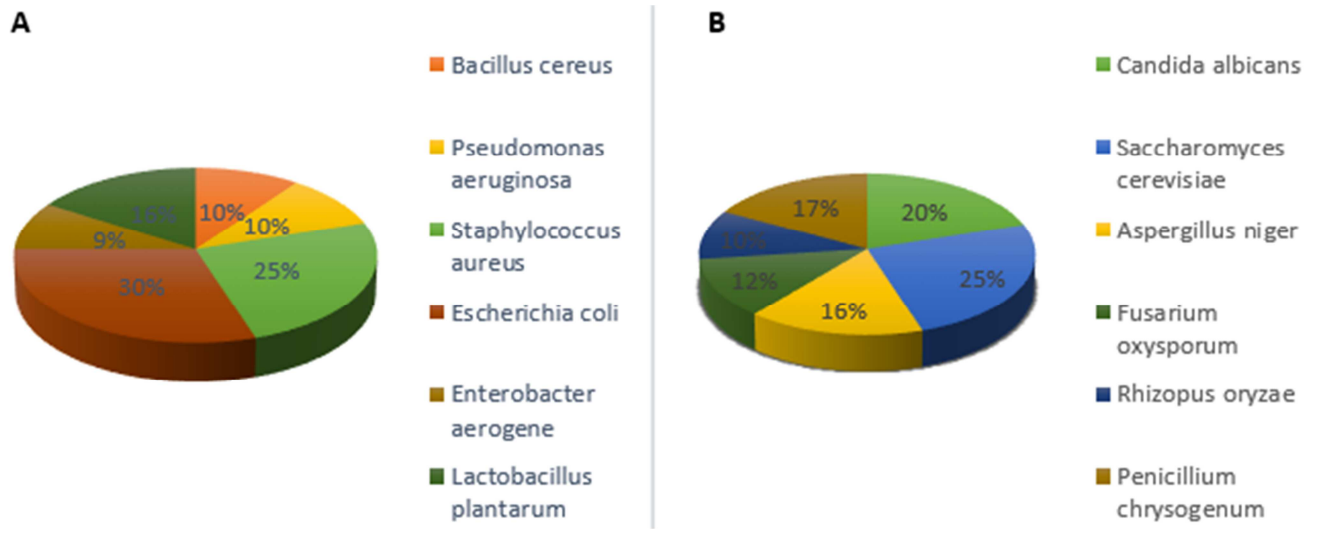

Figure 3. *Pie chart showing percentage of the microbial isolates from the fufu sold in the sample markets of Benin City. (A) Bacterial prevalence of fufu sold in Benin City with Escherichia coli being the most prevalence and Enterobacter aerogene the least prevalence. (B) Fungal prevalence of fufu sold in Benin City with S. cerevisiae the most prevalence and Rhizopus oryzae the least prevalence.*

4. Discussion

The fermentation of cassava roots, allows softening for further processing and reduction of potentially toxic cyanogenic glucosides present in the roots (Achi and Akomas, 2006). Submerged fermentation by traditional methods usually produce mash which contain a foul odour resulting from uncontrolled fermentation and storage techniques (Achi and Akomas, 2006; Okolie *et al.*, 1992). This type of fermentation, although the simplest way to achieve *fufu* through cassava fermentation, involves a complex microbial process according to Ogumbawo *et al*., 2004. Presence of these organisms could be as a result of post processing contamination from poor handling 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 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 have delight in consuming it. Reduction of the odour in *fufu* by enhance technology (optimization) will makes it to be more liked and generally acceptable to a wider population. Cassava is one of the very few tropical crops where cyanide content has not restricted its use as an important staple food for human consumption. This is because a variety of processing technique have been developed in different parts of the world to make *fufu* more palatable (Aweke *et al*., 2012). The degree of reduction of cyanide in the final product varies greatly with the type of processing techniques used (Nhassico *et al*., 2008; Cardoso *et al*., 2005; Bradbury, 2004). As the plant has long history of cultivation and consumption in other parts of the world, different processing methods had developed to neutralize the toxin (cyanide). Few processing methods such as washing, boiling, drying and fermenting with cereals are used to remove or reduce cyanide in cassava (Aweke *et al*., 2012). Solar drying and fermentation were found to be the best methods in removing cyanide content and detoxifying cassava based foods (Aweke *et al*., 2012).

5. Conclusion

The results from this research shows that although *fufu* is a fermented product, there is possibility of post contamination resulting from poor handling either at the processing site or from the market place. Quality is often compromised as some processors make use of back slopping to reduce the length of time for fermentation so as to meet high customers' demand and high quantity. The result obtained from this study showed that the *fufu* sold at the various sample markets in Benin City is contaminated with several microorganisms and might not be fit for consumption. This study has therefore given insight into the Microbiological quality of *fufu* sold Benin City, Nigeria.

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