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Microbiological Quality Assessment of Pupuru and Plantain Flours in an Urban Market in Akure, Ondo State, South Western Nigeria

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Abstract

Cassava and plantain flours are commonly used in preparation of a variety of food delicacies in South Western Nigeria. These are however sold in open air markets, and are scarcely required to be subjected to safety checks by food safety authorities. A study was designed to assess the microbiological food quality of cassava (pupuru) and plantain flour purchased from different vendors at Oja-Oba, Akure, Ondo state, as well as the antimicrobial profile of microorganisms identified. Microbiological analyses of sixty (60) food samples were carried out. Results showed highest bacterial counts in plantain flour sample F4 (3.0 \times 10⁴ cfu/ml) and least for F1 (0.1 \times 10⁴ cfu/ml). Fungal count was highest $(3.1 \times 10^4 \text{ sfu/ml})$ in plantain flour F4; and least in plantain flour samples F5, F10, F12 with fungal count of 0.5×10^2 sfu/ml each. Different samples contained Enterobacter sp., Aeromonas sp., Klebsiella sp., Acinetobacter sp., Campylobacter sp., Corynebacterium sp. and Bacillus subtilis. Fungal isolates include *Penicillium crustosum* and *P. chrysogenum, Rhizopus* oryzae and Aspergillus niger. Percentage occurrence of isolates includes Enterobacter sp. (0.99%), Klebsiella sp. (91.12%), Acinetobacter sp. (1.97%), Campylobacter sp. (4.61%), Corynebacterium sp. (0.33%), and Bacillus subtilis (0.99%). Most predominant mould was Aspergillus niger, with a percentage occurrence of 45.5%; and least, Penicillium crustosum (4.5%). Gram positive bacteria showed resistance to cotrimoxazole and ceftriaxone; and Gram negative bacteria to zimnacef. The microbial isolates from these flours could cause different food intoxication and illnesses in humans. While prevailing unfavorable environmental conditions and food form may contribute to their presence in samples; their presence is unacceptable and counts need to be kept minimal for consumer safety. Local authorities need to carry out continued vendor education campaigns, and regular quality checks to assess their safety for consumption.

Subject Areas

Microbiology

Keywords

Pupuru, Plantain, Flour, Food Quality, Antimicrobial, Nigeria

1. Introduction

Microorganisms are microscopically small forms of life mainly organised as single-cell organisms while some may occur multicellular. They ubiquitous in the natural environment (water, soil, air etc.), and can naturally be found in foods or on the surfaces of foods as contaminants during the manufacturing process of food products [1]. Food fermentation is the process for producing ATP using endogenous organic compounds as both electron donors and acceptors. The chemical process of fermentation makes a few ATP molecules in the absence of aerobic respiration [2]. Cassava flour (pupuru) is a fermented cassava meal that is consumed by at least 4 - 6 million people in Nigeria [3]. Plantain flour is the product of dried and pulverised plantain slices. Plantain is a major source of food in many regions throughout Nigeria and sub-Saharan African. Plantain production reaches over 80 million tonnes per year [4]. Plantain flour apart from being used as a substitute for garri especially for diabetic patient, also serves as raw material used in the production of cakes, puff-puff, biscuit, bread and pancakes. Plantain flour is a cheap source of iron, protein, vitamin C (3.0% to 3.5%), and carotene. It could be used in formulating protein supplement diets for both children and adults. The low level of sodium in plantain also makes it a ready source of raw material for formulating low sodium diets [4]. Traditional carbohydrate food such as cassava flour (pupuru) plays a key role in African diet. However, the production processes and sale condition leaves much to be desired as these flours are prone to microbial contamination which may cause food borne illnesses [5].

Food borne illnesses result from consumption of pathogenic bacteria, viruses or parasites that contaminate food, as well as chemical or natural toxins such as those in poisonous mushrooms. The consumption of toxins or microorganisms may cause food poisonings (intoxications) or infections. Contaminating microorganisms enter foods from a variety of sources. Crops carry soil borne bacterial species to the processing plant, and rodents and arthropods transport microorganisms on their feet and body parts as they move about among foods. Human handling of foods also provides a source of contamination. Factors that determine if spoilage will occur include water activity/moisture content of the food stuff, pH, physical structure of the food, presence/absence of oxygen, temperature of storage, food chemical composition [6]. More than 250 different food

borne diseases have been described [7]. Center for Disease Control and Prevention (CDC) estimated that 9.4 million of the illnesses caused by 31 known food borne pathogens, and that 90% of all illnesses due to known pathogen are caused by seven pathogens: *Salmonella, Norovirus, Campylobacter, Toxoplasma, E. coli* 0157:H7, *Listeria* and *Clostridium perfringens* [7]. All food borne diseases are associated with poor hygiene practices whether by water or food transmission or through faecal-oral route [8]. Often spoilage microorganisms are introduced from the same source as the food; others are introduced as contaminants during transport, storage or preparation. While observable changes to foodstuff are only likely after the microbial population has reached a considerable size, food poisoning can result from the presence of much smaller number of contaminants [9].

Locally, rural women process cassava into pupuru by steeping peeled cassava tubers in stream water and fermenting it for 4 - 6 days. This method of processing fouls the water and increases the level of microbial contaminants in the fermented cassava product [10]. The control and monitoring of conditions in the traditional processing methods are difficult. Another major constraint in the traditional processing of pupuru is the unduly time, as a result of ineffective heat transfer mechanism using the traditional dryer and probably due to the irregular and large sizes of the ball dried. Therefore, proper drying is not achieved and dried balls have the high moisture content, thus the product is susceptible to mold attack as well as developing some off flavor on storage. Also, prolonged drying may institute some changes that could negatively affect some functional properties of the product [11]. Presently in Ondo state of Nigeria, "pupuru" flour is often packaged in polyvinyl chloride container (covered plastic) and stored at ambient temperature (30°C ± 3°C). This packaging material does not protect the "pupuru" flour properly from contamination by insect, pests, microbes, dust and environmental moisture [12]. During the entire sequence of food handling, from the producer to the final consumer, microorganisms can affect food quality and human health. Contamination by disease-causing microorganisms can occur at any point in the food-handling sequence [13] [14]. Sources of microorganisms in food include food utensils, food handlers and animal hides [15].

Food borne illness is an international health problem that is common in most developing countries like Nigeria and has led to the death of thousands especially children with low immunity [7]. Screening for microorganisms in pupuru and plantain flour was undertaken to identify possible pathogens and contaminants, determine microbial load and antimicrobial susceptibility pattern of the microbial isolates. Information provided would increase our knowledge of the microorganisms associated with these foods and help in the development of appropriate management protocols to prevent and control associated food borne diseases. Reasons would also be suggested for the comparative distribution of the isolated microbes in the different food samples.

2. Materials and Methods

2.1. Samples Collection

A total of sixty food samples (thirty pupuru and thirty plantain flour) purchased from traders in Oja-Oba, Akure Ondo State were used for the study. The samples were collected in sterile polythene bags and transported to the laboratory for microbial analysis [16].

2.2. Sample Analysis for Microbial Isolation

Using the pour plate method of Aruwa and Akinyosoye [16], the microbial quality of the food sample was determined. About 1 g of each food sample was weighed aseptically into test tubes containing 9 ml of sterilized distilled water. Afterwards, the test tubes were shaken vigorously to allow dislodgement of food sample and even distribution of microorganisms. A four-fold serial dilution of each sample was prepared. 1ml of dilution factor 10^{-2} and 10^{-4} were inoculated into sterile Petri dishes containing nutrient agar (NA) and potato dextrose agar (PDA) for bacterial and fungal isolation/growth respectively. Incubation was carried out at 37° C for 24 hours for bacterial growth, and 25° C for 48-72 hrs for fungal growth.

2.3. Enumeration and Purification of Microbial Isolates

Colonies were counted at end of incubation period to obtain the total viable count. Calculation of colony forming unit (cfu) per ml for the bacteria and the spore forming unit (sfu) per ml for the fungi was done. Distinct colonies were sub-cultured from countable plates using the four-quadrant streak method to obtain pure bacterial cultures. The pure cultures of the bacteria isolated were each maintained in nutrient agar slants and refrigerated at 4°C. Fungal isolates were purified by the transfer of fungal mycelia plug onto sterile freshly prepared PDA plates. Pure cultures of the fungi isolated were maintained on PDA slants in McCartney bottles and kept in the refrigerator at 4°C [17].

2.4. Identification of Bacterial and Fungal Isolates

This was carried out according to the method of Cheesbrough [18] and Leboffe and Pierce [19] [20] [21] [22]. Gram's staining was carried out to ascertain the morphology and Gram's reaction of the isolates. Other biochemical tests carried out include catalase, coagulase, urease, spore staining and sugar fermentation. Fungal isolates were identified using microscopic and macroscopic mycelial morphologies.

2.5. Antibiotics Susceptibility Assay

In order to determine the susceptibility profile of isolates to clinically relevant antibiotics, the plate diffusion technique of Willey *et al.* [23] was employed. 18 - 24-hour old cultures of the isolated bacterial microorganisms were swabbed on sterile, solidified Muller Hilton agar (MHA) plates using sterile swab sticks. The

multiple antibiotic discs were then placed on the agar surface and pressed using sterile forceps to ensure complete contact with agar. All the plates were incubated at 37 °C to 24 hrs. The zone of inhibition was measured at the point which an obvious demarcation between growth and no growth could be seen using a meter rule. The antibiotics used and their corresponding concentrations were pefloxacin (10 μ g), chloramphenicol (30 μ g), gentamycin (10 μ g), ampiclox (30 μ g), zimnacef (20 μ g), amoxacillin (30 μ g), ciprofloxacin (10 μ g), streptomycin (30 μ g), septrin (30 μ g), erythromycin (10 μ g), rocephin (25 μ g), cotrimoxazole (25 μ g) for Gram positive bacteria; and streptomycin (30 μ g), chloramphenicol (30 μ g), gentamycin (10 μ g), cotrimoxazole (25 μ g), ofloxacin (5 μ g), amoxicillin (25 μ g), ciprofloxacin (10 μ g), erythromycin (5 μ g), pefloxacin (5 μ g) and ceftriazone (30 μ g) for Gram negative bacteria.

2.6. Statistical Analysis

Descriptive analyses were done with prevalence statistics expressed as percentages; and one-way ANOVA (SPSS version 15) for separation of means and determination of significant relationships with the p set at 0.05 significance level [16].

3. Results

The total bacteria count and total fungi count from food samples analysed was presented in **Table 1**. Highest bacterial count was found in plantain flour sample F4 with 3.0×10^4 cfu/ml, and lowest in F1 with 0.1×10^4 cfu/ml. The total fungi count was highest $(3.1 \times 10^4 \text{ sfu/ml})$ in plantain flour F4 while the least count was observed in plantain flour F5, F10, and F12 with fungal count of 0.5×10^2 sfu/ml. The identities of the bacteria isolated after colonial morphology, direct microscopic examination using Gram stain, and biochemical tests is presented in **Table 2**. The bacteria identified include *Enterobacter* sp., *Aeromonas* sp., *Klebsiella* sp., *Acinetobacter* sp., *Campylobacter* sp., *Corynebacterium* sp. and *Bacillus* sp. The fungi identified include *Penicillium* sp., *Rhizopus* sp., *Aspergillus* sp. (**Table 3**).

Klebsiella sp. bacterium was the most prevalent in both pupuru and plantain flour with a percentage prevalence of 72.9% and 94.5% (Table 4) respectively. The least prevalent among the bacteria isolated were Enterobacter sp. with prevalence of 6.3% in pupuru and Campylobacter sp. with 5.1% prevalence in plantain flour. Penicillium crustosum fungus was the most prevalent (66.7%), while Penicillium chrysogenum was the least prevalent (11.1%) in pupuru (Table 5). In plantain flour, Aspergillus niger was the most prevalent (61.5%) and the least prevalence was Rhizopus oryzae with prevalence of 38.5%.

Inhibition zones for Gram negative and Gram positive bacteria were presented in **Table 6** and **Table 7** respectively. Zones of inhibition for different antibiotics against Gram negative bacteria ranged from 11 - 29 mm, with the least zone of inhibition recorded for ciprofloxacin against *Klebsiella* sp. (11 mm) and

Table 1. Mean bacterial and fungal count in pupuru and plantain flour samples.

Pupuru flour samples	Bacterial count (cfu/ml)	Fungal count (sfu/ml)	Plantain flour samples	Bacterial count (cfu/ml)	Fungal count (sfu/ml)
P1	1.1×10^{4}	2.5×10^{4}	F1	0.1×10^4	0.5×10^4
P2	1.1×10^4	0	F2	0	5.5×10^2
Р3	0	0.5×10^4	F3	1.9×10^4	1.0×10^2
P4	1.1×10^4	4.0×10^4	F4	3.0×10^4	3.1×10^4
P5	0.1×10^4	1.0×10^2	F5	4.2×10^4	0.5×10^2
P6	1.5×10^4	0.5×10^4	F6	0.5×10^4	1.5×10^4
P7	0.54×10^4	2.0×10^4	F7	1.5×10^4	0
P8	1.5×10^4	2.0×10^2	F8	1.0×10^4	0
P9	1.5×10^4	0.5×10^4	F9	4.5×10^2	2.0×10^4
P10	2.0×10^4	1.0×10^4	F10	3.5×10^2	0.5×10^2
P11	0.51×10^4	0	F11	2.0×10^4	0.5×10^4
P12	0	3.0×10^2	F12	3.7×10^4	0.5×10^2
P13	6.6×10^4	2.0×10^4	F13	3.0×10^4	1.5×10^4
P14	0.5×10^4	1.0×10^4	F14	7.7×10^4	0.5×10^4
P15	0.5×10^4	0.5×10^4	F15	0.5×10^4	0.5×10^{5}

Key: P1-15 = pupuru samples; F1-F15 = plantain flour samples, from different sellers in Oja Oba market.

Table 2. Microscopic and biochemical characteristics of isolated bacteria from flour samples.

Bacteria Isolate	A 1	A2	A3	A4	A 5	A6
Shape	Rod	Rod	Rod	Rod	Rod	Rod
Gram reaction	-ve	-ve	-ve	-ve	+ve	+ve
Catalase	+	+	+	+	+	+
Coagulase	_	_	_	+	+	_
Glucose	++	++	+++	_	-	-
Lactose	+++	+++	+++	+++	+++	+++
Sucrose	+++	++	+++	+++	+++	+++
Maltose	+++	+++	+++	+++	+++	+++
Fructose	++	++	+++	++	+++	++
Urease	Wu	Wu	Wu	Su	-	-
Endospore	_	-	-	-	-	+
Probable bacteria	Enterobacter sp.	Aeromonas sp.	Klebsiella sp.	Campylobacter sp.	Corynebacterium sp.	Bacillus subtilis

Key: + = Positive, ++ = acidic, - = Negative, +++ = very acidic, + = fairly acidic, A (1, 2, 3, 4, 5, 6) = Probable bacteria, Wu = weak urease, Su = strong urease.

Table 3. Morphology of fungi isolated from flour samples

Fungal Isolates	Morphological Characteristics	Suspected fungus
Isolate A	Dull green to grey green. Conidiophore end possess clusters of two or more branches.	Penicillium crustosum
Isolate B	Cotton white, became typically grayish to brown on surface. Rapid growth.	Rhizopus oryzae
Isolate C	It is cotton-like in texture. Began as white but later turned green.	Penicillium chrysogenum
Isolate D	Black, umbrella-like mycelium shape. No conidia, the conidiophore hang the spore at the top.	Aspergillus niger

Table 4. Prevalence of bacterial isolates from pupuru and plantain flours.

Bacteria isolate	Pupuru Prevalence	Percentage (%)	Plantain flour Prevalence	Percentage (%)
Enterobacter sp.	3	6.3	-	-
<i>Klebsiella</i> sp.	35	72.9	242	94.5
Acinetobacter sp.	6	12.5	-	-
Campylobacter sp.	1	2.1	13	5.1
Corynebacterium sp.	-	-	-	-
Bacillus subtilis	3	6.25	-	-
Total	48	100	256	100

Table 5. Prevalence of fungal isolates from pupuru and plantain flours.

Fungi isolate		Pupuru e percentage (%)	Plantain flour Prevalence percentage (%)		
Penicillium crustosum	6	66.7	-	-	
Rhizopus oryzae	-	-	5	38.5	
Penicillium chrysogenum	1	11.1	-	-	
Aspergillus niger	2	22.2	8	61.5	
Total	9	100	13	100	

the higher range recorded for ofloxacin against *Enterobacter* sp. (29 mm). Zones for Gram positive bacteria, the range was from 7 - 22.3 mm, with higher range recorded for pefloxacin (22.3 mm) against *Corynebacterium* sp., with the least recorded for pefloxacin against *Bacillus subtilis* (7 mm).

4. Discussion

Changes in microbiological quality of a food item determine its safety, acceptability, shelf stability and its fitness for consumption. The array of microorganisms detected in flour samples used in this study may be associated with contaminated packaging materials, water used during processing, sneezing and coughing by handlers and exposure of flours in an open market environment. Production of most traditional food is often associated with unhygienic prac-

Table 6. Antibiotics zone of inhibition for Gram negative bacteria.

Bacteria				Ant	ibiotic zone o	of inhibition	(mm)			
isolate	STR	CHL	GEN	COT	OFL	AMX	CPX	ERY	PFX	CRO
Enterobacter sp.	17.30 ± 0.50°	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	29.00 ± 1.00 ^f	0.00 ± 0.00^{a}	25.60 ± 0.50°	14.00 ± 1.00 ^b	23.00 ± 0.00 ^d	0.00 ± 0.00^{a}
<i>Klebsiella</i> sp.	0.00 ± 0.00^{a}	19.00 ± 1.00 ^{de}	17.60 ± 1.00°	0.00 ± 0.00^{a}	18.60 ± 0.50^{cd}	0.00 ± 0.00^{a}	11.00 ± 0.00^{b}	$17.60 \pm 0.50^{\circ}$	20.00 ± 1.00°	0.00 ± 0.00^{a}
Acinetobacter sp.	20.00 ± 1.00°	21.30 ± 1.00°	15.00 ± 1.00 ^t	$^{\circ}0.00 \pm 0.00^{a}$	25.00 ± 1.00^{d}	14.30 ± 0.50^{1}	°27.00 ± 0.00°	28.00 ± 1.00°	16.00 ± 1.00 ^b	0.00 ± 0.00^{a}
Campylobacte sp.	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	$17.60 \pm 0.50^{\circ}$	0.00 ± 0.00^{a}	17.30 ± 0.50^{b}	0.00 ± 0.00^{a}	19.30 ± 0.50 ^d	0.00 ± 0.00^{a}

Data are presented as Mean \pm S.E (n = 3). Values with the same superscript letter(s) along the same column are not significantly different (P > 0.05). **Key:** STR = streptomycin (30 μ g), CHL = chloramphenicol (30 μ g), GEN = gentamycin (10 μ g), COT = cotrimozazole (25 μ g), OFL = oflaxacin (5 μ g), AMX = amoxicillin (25 μ g), CPX = ciprofloxacin (10 μ g), ERY = erythromycin (5 μ g), PFX = pefloxacin (5 μ g), CRO = cetriazone (30 μ g).

Table 7. Antibiotics zone of inhibition for Gram positive bacteria.

Bacterial isolate				Antib	iotic zone of	inhibition (mm)			
	AM	R	CPX	S	SXT	Е	PEF	CN	APX	Z
Corynebacterium sp.	11.60 ± 0.50^{a}	20.30 ± 0.50 ^b	19.60 ± 0.05 ^b	20.00 ± 0.00 ^b	11.30 ± 1.00^{a}	20.00 ± 1.00 ¹	b 22.30 ± 0.50°	19.00 ± 1.00 ^b	19.60 ± 1.00 ^b	10.30 ± 1.00
Bacillus subtilis	0.00 ± 0.00^{a}	12.00 ± 1.00^{d}	7.00 ± 1.00^{b}	12.30 ± 0.50^{d}	0.00 ± 0.00^{a}	$9.6 \pm 0.50^{\circ}$	18.60 ± 0.50e	$20.30 \pm 0.5^{\rm f}$	0.00 ± 0.00^{a}	$0.00 \pm 0.00^{\circ}$

Data are presented as Mean \pm S.E (n = 3). Values with the same superscript letter(s) along the same column are not significantly different (P > 0.05). **Key:** AM = amoxicillin (25 μ g), R = rocephin (25 μ g), CPX = ciprofloxacin (10 μ g), S = streptomycin (30 μ g), SXT = septrin (30 μ g), E = erythromycin (10 μ g), PEF = pefloxacin (10 μ g), CN = gentamycin (10 μ g), APX = ampiclox (30 μ g), Z = zimnacef (20 μ g).

tices. This has been documented for these and other food items by Ohenhen et al. [24], Ogori and Gana [25] and Ojokoh et al. [26]. These food samples contain different bacteria that are opportunistic pathogens. If consumed by individuals with compromised immune systems they can cause several types of food borne diseases. Enterobacter sp., Klebsiella sp., Acinetobacter sp., Campylobacter sp., Corynebacterium sp., and Bacillus subtilis are resident and transient bacteria transferred respectively on hands and associated with poor hygiene practices. The high bacteria counts and high occurrence of Klebsiella sp. and presence of other bacteria in plantain and pupuru flours are indicative of potential public health hazard [25].

Penicillium crustosum, Rhizopus oryzae, Penicillium chrysogenum and Aspergillus nigerfungi which were isolated and identified are common moulds in soil, air and organic matter and are therefore ready contaminants of foods and feeds. Aspergillus sp. and Bacillus sp. are known to be associated with dried food [26]. These moulds are responsible for off flavours and taste in food samples, therefore resulting into food spoilage. Aspergillus spp. and Rhizopus spp. are indigenous to cassava fermentation and indication of public health hazard. A. niger is also known to produce mycotoxins (aflatoxins) [25]. The dominating presence of Aspergillus species on the sampled foods could be due to its rapid sporulation, development and relative adaptability to a wide variety of environmental conditions as compared to other species. It also indicates that unconditioned and unhygienic approaches were used in processing [25].

Aflatoxins are still recognized as the most important mycotoxins. They are synthesized by only a few Aspergillus species, of which Aspergillus flavus and Aspergillus niger are the most problematic. Obadina et al. [27] reported the presence of Aspergillus, Rhizopus and Penicillium species in other dried cassava products. Shittu et al. [28] also demonstrated the presence of toxigenic moulds like Aspergillus flavus and Penicillium species, which could constitute a health hazard to consumers and cause pupuru spoilage. Their presence and high counts in the flours sampled could be attributed to favourable environmental condition for their growth. The expression of aflatoxin-related diseases is influenced by factors such as age, nutrition, sex, species and the possibility of concurrent exposure to other toxins [29]. Also in this study, antimicrobial sensitivity test was carried out on microbial isolates and found to be of public health significance, having relatively varied antibiotic resistance profiles. Resistance or susceptibility of microbes to antimicrobial agents are determined by inherent microbial genetic and environmental factors. Some of these factors include kinds of microorganism present, the concentration and nature of the antimicrobial, and length of exposure to the agent etc. The wide antibiotic resistance profile of Campylobacter sp. and Bacillus subtilis is of concern as these microbes can cause food borne illnesses. Hence, the tested antibiotics would not be efficacious in the treatment of food borne diseases that may be caused by these microbes. Microorganisms like these may have acquired or developed the ability to resistance and virulence factors to protect themselves in harsh/dry environments. Such trends constitute significant health problems for the future [30].

5. Conclusion

The environment plays a critical role in the transmission of infectious agents to humans, with various materials serving as mechanical vehicles. Microbial contaminants may be transmitted directly through hand to hand contact or indirectly via food or other inanimate objects. The present study has focused on the microbiological quality of pupuru and plantain flour and potential health hazard that may arise from consumption of improperly processed flours. Results contribute to ensuring safe food product delivery to meet the demands of consumers now and in future. In order to ensure quality control; prevention of undue food contamination prior to processing, use of adequate and appropriate food processing techniques to avoid contamination during and after foods processing, quality packaging to keep foods fresh and minimize health risk factors, adequate storage, ideal transportation, and hygienic handling of the finished products cannot be overemphasized.

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