Research Journal of Microbiology

ISSN 1816-4935

Academic Journals Inc.

www.academicjournals.com

Research Journal of Microbiology 2 (11): 831-837, 2007
ISSN 1816-4935

Microbial Diversity in Ready-to-eat Fufu and Lafun-Fermented Cassava Products Sold in Ile-Ife, Nigeria

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Abstract: A microbiological study was undertaken to determine the microbial diversity and quality of RTE fufu and lafun sold in eateries in Ile-Ife, Nigeria. Twenty samples of RTE fufu and lafun were analysed for pH and microbial quality. The mean total mesophilic aerobic bacteria, lactic acid bacteria, Enterobacteriaceae, yeasts and Staphylococcal counts of the samples ranged from 3.44-4.79, 3.06-4.94, 3.41-4.75, <1-2.26 and 2.47-4.84 $\log_{10}$ cfu g^{-1} sample, respectively. The predominant bacteria were of the genera *Bacillus*, *Corynebacterium*, *Micrococcus*, *Staphylococcus*, *Salmonella*, *Klebsiella*, *Enterobacte*r, *Citrobacter*, *Lactococcus* and *Lactobacillus*. Yeasts were identified as species of *Candida*, *Saccharomyces* and *Debaryomyces*. The pH of the samples ranged from 3.65 to 5.12. The RTE lafun samples had a higher mean pH and microbial population and a wider variety of microorganisms than RTE fufu. This study has demonstrated the microbial diversity in RTE fufu and lafun sold in Ile-Ife, Nigeria. The presence of foodborne pathogens and unacceptable limits of enteric bacteria in these main dishes poses some potential risks to the consuming public. The need for improvement and maintenance of good hygienic practices by food handlers and vendors is emphasized.

Key words: Fermented cassava, fufu, lafun, microbial diversity

INTRODUCTION

Cassava (*Manihot esculenta* Crantz) is an important root crop in Africa, Asia, South America and India where it provides over 50% of the average daily colorific intake in some countries (de Bruijn and Fresco, 1989). Physiological deterioration of cassava roots occur 2-5 days after harvesting followed by microbial deterioration 3-5 days later (Nweke, 1994). Cassava tubers vary widely in their cyanogenic content with most varieties containing about 15 to 400 mg HCN per Kg fresh weight (Padmaja, 1995). Cassava root is processed before consumption in other to stabilize and detoxify it (Ampe and Brauman, 1995). Fermentation is an important processing method widely used to transform and preserve cassava because of its low technology and energy requirements and the unique organoleptic qualities of the final products (Ampe *et al*., 1994). Common fermented cassava products of West Africa include gari, fufu and lafun among others (Oyewole, 1991). The solid-state fermentation process produces Gari while the submerged fermentation process produces fufu and lafun (Oyewole and Odunfa, 1992). After fermentation, cassava is subjected to sun-drying and milled to produce lafun flour. Lafun flour is usually turned in boiled water with no extra heating and made to a stiff porridge (Oyewole and Afolami, 2001). In the case of fufu, fermented cassava roots are mashed and cooked into dough (Oyewole and Ogundele, 2001). The fermented cooked lafun and fufu are consumed with vegetable soups.

Most of the published work on cassava has focused on the detoxification of the cyanogenic glycosides (Ampe and Brauman, 1995), microorganisms involved in the fermentation (Brauman *et al*.,

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1996; Coulin *et al*., 2006), biochemical and extracellular enzyme activities accompanying the fermentation process (Oyewole and Odunfa, 1992; Brauman *et al*., 1996). Also, faecal contaminants and pathogenic bacteria have been reported in other ready-to-eat foods in Nigeria (Owhe-Ureghe *et al*., 1993), street foods in Accra, Ghana (Mensah *et al*., 2002) and Mangalore (Bhaskar *et al*., 2004). There appears to be no information on the microorganisms associated with fermented cooked Ready-to-eat (RTE) fufu and lafun (cassava products) in Nigeria. Fermented cooked RTE fufu and lafun are usually wrapped in small polythene bags, hawked alone or sold in eateries with accompanying vegetable soup in Nigeria. Most often, the cooked RTE lafun and fufu keep for 24 h and up to 72 h, respectively and can be eaten warm or cold. Since consumers cannot tell the risk of incurring food-borne illness at the time of purchase or consumption of food (especially ready -to-eat street foods), information on the safety of foods (especially microbiological) may help consumers avoid purchases and consumption of certain food items. The objective of the present study was therefore to examine the microbial composition of RTE fufu and lafun sold in some eateries in Ile-Ife, Nigeria to evaluate their microbiological quality.

MATERIALS AND METHODS

A study on the microbial diversity in ready-to-eat fufu and lafun sold in Ile - Ife was carried out in the Food Microbiology Laboratory, Department of Microbiology, Obafemi Awolowo University, Ile-Ife, Nigeria between June 2006 and January 2007.

Collection of Samples

Ten samples each of RTE fufu and lafun were purchased from different eateries in Ile-Ife, Nigeria. Samples were collected in sterile stomacher bags in icebox and transported immediately to the Food Microbiology laboratory for analysis.

pH Determination

Freshly distilled water was boiled to expel CO_2, cooled to room temperature and used to prepare 10^{-1} homogenate of the samples. Approximately 10 g of sample was homogenized with 90 mL of distilled water in a blender and the pH of the slurry was measured with a pH meter (Hanna Instruments, 8520).

Microbiological Analysis

Ten gram of sample was homogenized with 90 mL of sterile maximum recovery diluent (MRD, Oxoid CM 733) by stomaching for 2 min (Colworth Stomacher 400). Subsequent decimal dilutions were prepared in sterile MRD and appropriately diluted suspension of sample (1.0 mL) was mixed with molten (45°C) media and poured into sterile plates. Total Mesophilic Aerobic Bacteria (TMAB) were enumerated in pour-plates of plate count agar (PCA, Oxoid CM325) incubated at 30°C for 48 h. Yeasts were enumerated in pour-plates of sabouraud 4% glucose agar (Fluka) supplemented with chloramphenicol (100 mg L^{-1}) incubated aerobically at 28°C for 72 h. Lactic Acid Bacteria (LAB) were enumerated in pour-plates of de Man, Rogosa and Sharpe agar (Oxoid) incubated under anaerobic condition in candle jar at room temperature for 72 h. Selective enumeration of enteric bacteria was carried out in pour-plates of violet red bile glucose agar (Fluka) incubated at 35°C for 24-48 h. *Staphylococcus* species were enumerated in poured plates of mannitol salt agar (Himedia, MM118 India) incubated at 35°C for 24-48 h.

All colonies appearing at the end of the incubation period were counted and the results expressed as $\log_{10}$ cfu g^{-1} sample. Colonies of bacteria and yeast developing on plates were observed, isolated and purified by repeated streaking on fresh agar plates of the isolation media.

The purified bacteria isolates were characterized and identified following standard methods (Harrigan and McCance, 1976; Sneath *et al*., 1986; Collins *et al*., 2004). Yeast isolates were characterized and identified as described by Lodder (1970) and Van der Walt and Yarrow (1984).

The data on microbial counts and pH were subjected to statistical analysis using Primer for Biostatistics software package version 3.01 (Glantz, 1992). Statistical significance was accepted at p-value equal to or less than 0.05.

RESULTS AND DISCUSSION

The fufu samples had a mean pH of 3.78 while lafun samples had a mean pH of 4.26 (Table 1). The acidic pH value of the RTE samples was not unexpected since cassava is known to undergo acid fermentation during the steeping stage of fufu and lafun production (Oyewole and Odunfa, 1988; Oyewole and Ogundele, 2001). The pH value of the RTE fufu and lafun falls within the pH range reported for fermented uncooked cassava tissue (Achi and Akomas, 2006).

The RTE fufu and lafun samples contain a wide variety of microorganisms (Table 1). The mean total mesophilic aerobic bacteria count obtained for most of the samples was within the acceptable limits of $< 5.0 \log_{10}$ cfu g^{-1} while the levels of Enterobacteriaceace was above the acceptable limits of $< 3.0 \log_{10}$ cfu g^{-1} sample. The mean Staphylococci counts ranged between 2.47 and 4.84 $\log_{10}$ cfu g^{-1} in fufu and lafun, respectively. The Staphylococci counts was less than 5.0 $\log_{10}$ cfu g^{-1} sample ruling out the possibility of *Staphylococcus aureus* food poisoning resulting from their consumption (Garbutt, 1997). The mean lactic acid bacteria count was 3.06 and 4.94 $\log_{10}$ cfu g^{-1} in fufu and lafun, respectively. Yeasts count was either very low ($< 1.0 \log_{10}$ cfu g^{-1}) or was not detected in some fufu samples while the lafun samples had a mean yeast count of 2.26 $\log_{10}$ cfu g^{-1} sample.

The acidic pH value of the RTE fufu and lafun samples may have restricted the growth of certain microorganisms thereby resulting in a rather lower microbial count compared to counts obtained in other RTE food products (Owhe-Ureghe *et al*., 1993; Alonso-Calleja *et al*., 2004; Thapa *et al*., 2004). It is significant to note that our mean counts for total mesophilic aerobic bacteria, Enterobacteriaciceae and staphylococcal in RTE fufu samples were lower than counts obtained for RTE fufu samples in Accra, Ghana (Mensah *et al*., 2002). The method of preparation, handling and environmental factors may have being responsible for the observed difference in microbial load. Present findings on microbial load of RTE lafun could not be related to other reports as this is to the best of our knowledge the first report on microbiological examination of RTE lafun. The slightly higher pH value of lafun samples may have created a slightly favourable condition for the growth of microorganisms hence the observed higher microbial load than in fufu samples. Furthermore, the method of preparing lafun flour into RTE lafun (flour is turned in boiled water to a stiff porridge with no extra heating) may not have eliminated some of the microorganisms introduced into the flour during the sun-drying stage of its production. In addition, the boiled water used in the preparation of the stiff lafun porridge and handling during the wrapping of the product in small polythene bags may have contributed to the microbial load. The lower microbial load of fufu over lafun samples was probably a reflection of the heating process involved in the cooking of fermented mashed cassava root into RTE fufu.

The lactic acid bacteria consistently isolated from RTE fufu and lafun were *Lactococcus plantarum*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus fermentum* (Table 2). Other bacteria species isolated were of the genera *Bacillus*, *Corynebacterium*, *Propionibacterium*, *Micrococcus*, *Staphylococcus*, *Shigella*, *Salmonella*, *Klebsiella*, *Citrobacter* and *Enterobacter*. The Yeast species isolated were *Candida* sp. *Debaryomyces* sp. and *Saccharomyces* sp. (Table 3). Some of the microbial isolates were not detected in fufu samples while lafun samples contain all the isolates (Table 4). Of significant note is the absence of *Shigella* sp. an agent of diarrhea in the RTE fufu

Table 1: pH and microbial counts of ready-to-eat fufu and lafun

	Product	
Variable	Fufu	Lafun
pH	3.78±0.04[a]	4.26±0.24[a]
Microbial count*		
TMAB	3.44±0.20[b]	4.79±0.46[c]
LAB	3.06±0.29[a]	4.94±1.02[a]
Yeasts	< 1***[a]	2.26±0.32[b]
Enterobacteriaceae	3.41±0.57[a]	4.75±0.44[a]
Staphylococcal	2.47±0.27[b]	4.84±0.43[c]

Data represent means±standard error (n = 10). Means bearing different superscripts within each row differ significantly ($p<0.05$). Microbial count expressed as $\log_{10}$ cfu g^{-1} sample.**, not detected in some samples

Table 2: Characteristics of lactic acid bacteria strains isolated from ready-to-eat fufu and lafun

	Isolate code				
Characteristics	LA	LB	LC	LD	LE
Cell morphology	Coccus	Rod	Rod	Coccus	Rod
Gram rxn	+	+	+	+	+
Catalase	–	–	–	–	–
Growth at					
15°C	–	–	+	+	–
45°C	–	+	+	–	+
CO_2 from glucose		–	–	+	+
Arginine hydrolysis	–	–	+	+	
Sugar fermentation					
Salicin	–	–	+	+	+
Ribose	–	–	–	+	+
Sucrose	–	–	–	+	+
Arabinose	–	–	–	+	+
Melibiose	–	–	–	+	+
Lactose	–	+	–	+	+
Sorbitol	–	–	–	+	–
Xylose	–	+	–	+	+
Maltose	+	–	–	–	–
Cellobiose	–	–	+	+	–
Raffinose	–	+	–	+	+
Trehalose	–	–	–	+	–
Rhamnose	–	–	–	+	+
Melezitose	–	–	–	+	+
Mannitol	–	–	–	+	+
Probable identity	*Lactococcus plantarum*	*Lactobacillus bulgaricus*	*Lactobacillus casei*	*Lactococcus* sp	*Lactobacillus fermentum*

+: Positive; -: Negative

samples studied. The presence of a wide variety of microorganisms in the lafun samples may be a reflection of the unsanitary traditional method of drying the pulverized fermented cassava tissue on the road side, grinding into lafun flour and subsequent inadequate heating process involved in the preparation into RTE product.

Of the microorganisms isolated from the RTE fufu and lafun samples, *Bacillus* sp. *Klebsiella* sp. *Lactobacillus* sp. *Corynebacterium* sp. and *Candida* sp. have all been reported as predominant microflora of fermenting cassava for fufu and lafun production (Oyewole and Odunfa, 1988; Achi and Akomas, 2006). In a similar study on RTE street foods in Accra, Ghana, Mensah *et al*. (2002) reported *Citrobacter* sp. *Enterobacter* sp. *Staphylococcus aureus* and *Bacillus* sp. in fufu samples. *Salmonella* sp. *Shigella* sp. lactic acid bacteria, *Corynebacterium* sp. *Micrococcus* sp. *Klebsiella* sp. *Enterobacter* sp., has all been reported in other RTE street foods (Owhe-Ureghe *et al*., 1993; Mensah *et al*., 2002; Bhaskar *et al*., 2004).

The presence of enteric bacteria above the acceptable limits in the fufu and lafun samples make them microbiologically unacceptable for consumption and this indicate faecal contamination resulting

Table 3: Characteristics of yeasts isolated from ready-to-eat fufu and lafun

	Isolate code		
Characteristics	YA	YB	YC
Cell morphology	Ovoid	Spherical	Ovoid
Mycelium	Pseudo	–	Pseudo
Spore*	–	–	–
Nitrate reduction	+	–	–
Sugar fermentation			
Glucose	+	+	+
Galactose	–	–	–
Raffinose	–	–	+
Sucrose	+	–	+
Fructose	+	+	+
Lactose	–	–	–
Maltose	–	–	–
Sugar assimilation			
Lactose	+	+	–
Maltose	–	–	+
Sucrose	+	–	+
Raffinose	+	–	–
Galactose	+	–	+
Glucose	+	+	+
Probable identity	*Debaryomyces* sp.	*Saccharomyces* sp.	*Candida* sp.

*Spore on Gorodkowa agar, +: Positive; –: Negative

Table 4: Frequency of isolation of microbial species from ready-to-eat fufu and lafun

	Ready-to-eat fermented cassava products	
Microbial isolates	Fufu	Lafun
Bacillus subtilis	+ (80)	+(100)
B. pumilus	+ (70)	+ (80)
B. cereus	+ (20)	+ (20)
B. macerans	-(0)	+ (40)
B. circulans	-(0)	+ (40)
Corynebacterium sp.	+ (40)	+ (60)
Propionibacterium sp.	+ (20)	+ (20)
Micrococcus varian	+ (60)	+ (60)
Staphylococcus aureus	+ (70)	+ (70)
Staphylococcus sp.	+ (40)	+ (30)
Salmonella sp.	+ (60)	+ (40)
Shigella sp.	-(0)	+ (40)
Klebsiella sp.	+ (20)	+ (40)
Citrobacter freundii	+ (20)	+ (40)
Enterobacter aerogenes	+ (20)	+ (60)
Lactococcus sp.	– (0)	+ (60)
L. plantarum	+ (40)	+ (20)
Lactobacillus casei	+ (40)	+ (20)
L. bulgaricus	+ (40)	+ (20)
L. fermentum	+ (40)	+ (40)
Candida sp.	+ (20)	+ (40)
Dabaryomyces sp.	– (0)	+ (40)
Saccharomyces sp.	– (0)	+ (40)

–: Not detected +: Detected, Percentage occurrence of the microbial species in the samples is shown in parentheses

from inadequate processing and or post process recontamination from flies, dirty wrapping material or poor hygienic handling. In a similar investigation, Mensah *et al.* (2002) also reported enteric bacteria level above the acceptable limit in RTE street fufu samples in Accra, Ghana. The presence of these enteric bacteria in the food samples poses a potential health concern to the consuming public. The presence of *Staphylococcus aureus* is suggestive of contamination from the skin, mouth and nose of the food handlers, which is an indication of poor personal hygiene. The lactic acid bacteria present in the RTE fufu and lafun samples ranged from species of coccal- lactics to species of homofermentative

and heterofermentative rods. Under the prevailing ambient temperature of storage of the RTE samples while still on sale, the lactic acid bacteria would grow, increase in their numbers and produce acid that may be antagonistic to some contaminating microorganisms in the food. However, higher levels of lactic acid bacteria may result in spoilage (sour and acid odour) of the RTE foods kept beyond 24 h at ambient temperature. Street foods (in countries with high ambient temperature conditions) held for a sufficient length of time has been reported to encourage proliferation of bacteria that may lead to spoilage and food illness (Van Steenberger *et al*., 1983; Bryan and Bartleson, 1985). The presence of *Bacillus* species in the RTE fufu and lafun was not unexpected since the food samples may have been contaminated by *Bacillus* endospores from dust especially since the RTE foods are handled at ground level. The presence of *Bacillus cereus* in about 20% of the RTE samples studied may constitute a health risk since *B. cereus* is known to be associated with food infection. Although, selective *Bacillus cereus* agar base was not used to enumerate the level of *B. cereus* in the samples, a small number of *B. cereus* counts in foods have been reported to be insignificant (Beumer, 2001).

The data obtained in this study has demonstrated the microbial diversity in RTE fufu and lafun sold in Ile-Ife, Nigeria and confirms previous microbiological reports on other ready-to-eat street foods (Owhe-Ureghe *et al*., 1993; Mensah *et al*., 2002; Bhaskar *et al*., 2004). Although the mean total mesophilic aerobic bacteria and staphylococcal counts of the RTE fufu and lafun are within the acceptable range, the presence of unacceptable limits of enteric bacteria and some pathogenic contaminants in some of the samples make them microbiologically unacceptable and indicate the need for urgent improvement on the hygienic condition of street RTE foods. Further investigation on the risk factors that could predispose RTE fufu and lafun to microbial contamination would assist in the education of food vendors/handlers on how to improve the microbial quality of RTE street foods. Special attention should be given to the effective communication on microbiological food risk, proper instruction on handling procedures during and after cooking food, consumer/vendor education on transmission of food borne diseases and more vigilant monitoring by public health authorities (Food inspectors and control staff). Actions along these lines in addition to serving food hot can be expected to improve the safety of RTE street foods.

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