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Public health issues in the processing of cassava (*Manihot esculenta*) for the production of *lafun* and the application of hazard analysis control measures

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RESEARCH ARTICLE

Abstract

Eight hundred samples of water, fermenting broths and dried *lafun* obtained from processors from two villages in Ogbomoso, Nigeria were analysed within a period of five months for microbiological attributes through the enumeration and isolation of mould/yeast, mesophilic aerobic bacteria, coliform, staphylococci, *Salmonella* and lactobacilli. In addition, the public health implications of the incidence of bacteria and fungi were investigated through antibiotic sensitivity testing and determination of aflatoxigenic potential of fungal isolates. The raw materials and environments in which *lafun* processing was carried out was observed to identify sources of hazards associated with the production process, to construct a workable hazard analysis and critical control points plan for *lafun* production, which was implemented in the production of laboratory-prepared *lafun.* The cumulative microbial counts of the water and fermenting broths ranged from 1.35×10^4 to 1.04×10^5 cfu/ml, while that of *lafun* samples ranged from 2.21×10^4 to 9.91×10^4 cfu/g. There were occurrences of *Staphylococcus aureus*, *Escherichia coli*, *Salmonella* Typhimurium, *Lactobacillus* sp., *Bacillus cereus*, *Klebsiella oxytoca*, *Aspergillus fumigatus*, *Aspergillus flavus*, *Aspergillus niger*, *Absidia corymbifera* and *Rhizopus oryzae* in the samples. Many of these isolates showed multi-drug resistance to 2-10 antibiotics, while about 39% of the fungi produced aflatoxins ranging from 1 to 1,600 μg/kg. The critical control points identified in the production were steeping, drying, packaging/storage, and the implementation of the corrective measures led to the production of laboratory-prepared *lafun* with improved microbiological safety. The results showed potential hazards that may be associated with the consumption of *lafun* with attendant public health consequences of aflatoxin food poisoning and illnesses that may be occasioned by multi-drug resistant bacteria. Education of processors on the hazards, critical control points and importance of hygienic environment is therefore necessary in the production of quality *lafun* to safeguard public health.

Keywords: aflatoxin, antibiotic resistance, fermentation, food safety, HACCP

1. Introduction

Cassava (*Manihot esculenta*) is one of the major staple food plants in the World, utilised by nearly a billion people in 105 countries from tropical Africa, Asia and Latin America (FAO, 2004). It is a perennial woody shrub that produces tuberous edible roots that can be processed into various forms of important food items such as *fufu*, *gari* and *lafun* (Ogiehor *et al.*, 2007; Oyewole and Ogundele 2001; Padonou *et al.*, 2009). The processing ensures detoxification, preservation and modification of the fermented cassava products (Oyewole, 1991). *Lafun* is produced through the submerged fermentation of peeled cassava roots in water (Oyewole and Odunfa, 1988). After fermentation, the fermented cassava is subjected to sun-drying and milled in order to have *lafun* flour (Figure 1). The flour is usually turned in boiled water, with no extra heating and made to a stiff porridge which is consumed with soup. It is similar to *cossettes* in Congo and Rwanda, *kanyanga* and *mapanga* in Malawi and *makopa* in Tanzania (Evans *et al.*, 2013).

Cassava production in Africa is used almost exclusively for consumption as food (Ogbe *et al.*, 2007). In fact, 95% of the total cassava production, after accounting for waste,

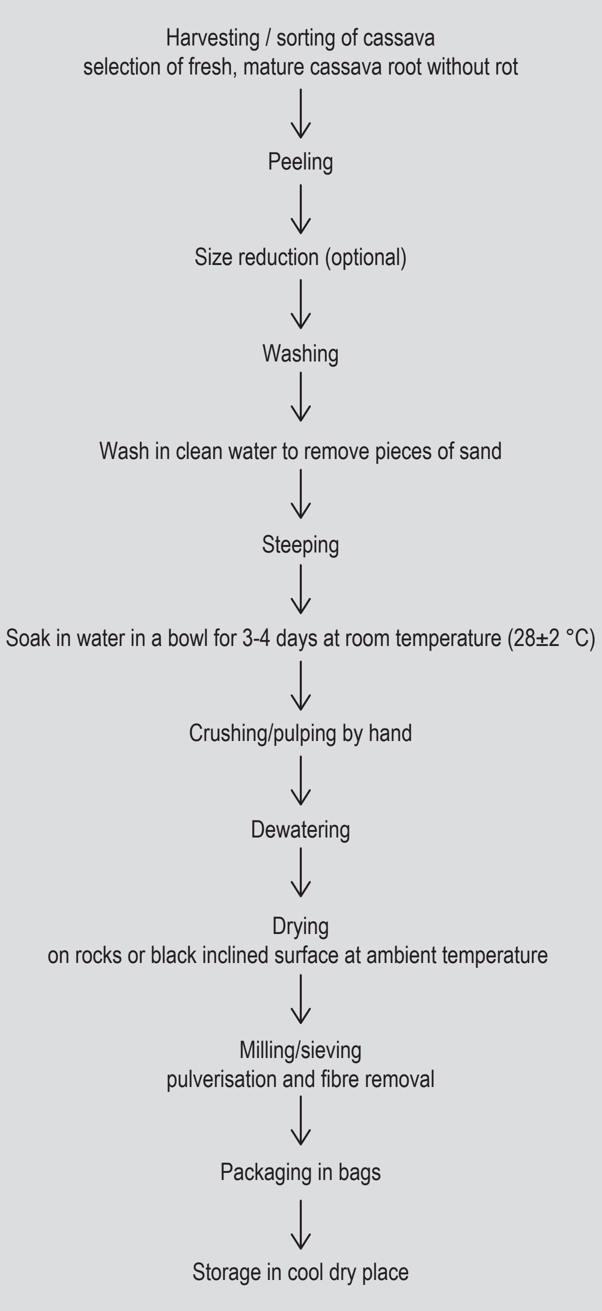

Figure 1. Process diagram for the production of *lafun* (Oyewole and Sanni, 1995; Padonou *et al.*, 2009).

was used as food in Africa in the late 1990s, and the total cassava consumption more than doubled from 24 million tons per year in the early 1960s to 58 million tons per year in the late 1990s (FAOSTAT, 2002). According to FAO (2008), cassava is the developing world's fourth most important crop after wheat, rice and maize. In the year 2008, the estimated global production was 232, 950, 180 tons, with Nigeria ranked as world's largest producer.

The production process of cassava flour (*lafun*) is mostly carried out under unhygienic condition and the raw cassava is also contaminated with microorganisms in the soil. In addition, the crop can have deadly consequences if prepared incorrectly since the cassava plant can produce cyanide, a deadly compound which can cause vomiting, stomach pains, dizziness, headache, weakness and charkha when consumed. Chronic cyanide toxicity is also associated with several pathological conditions including konzo, an irresistible paralysis of the legs which has been reported in eastern, central and southern Africa (Evans *et al.*, 2013). Mycotoxins are also able to contaminate a wide-range of food commodities, including raw materials such as cassava or cereal-based products (Roscoe *et al.*, 2008). The staple commodities such as cassava, corn, wheat and a variety of spices intended for human or animal consumption have been reported to be regularly contaminated with aflatoxins (Wild and Hall, 2000). The incidence of food-borne pathogens such as *Listeria* spp., *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Klebsiella* spp., and *Clostridium* spp. in samples of *lafun* have been documented (Adebayo-Oyetoro *et al.*, 2013; Ijabadeniyi, 2007; Obadina *et al.*, 2009). Although there is lack of reliable data on food-borne illnesses in the country, the presence of the pathogens portends serious danger to the public health, as they contribute to the morbidity and mortality rates in the country (Omojokun, 2013). Therefore, there is the need to improve on the production techniques of food, especially the fermented food products in order to ensure safety of consumers.

As a result, this study was conceived to assess the hazards associated with the production of *lafun* among local producers over extended period of five months. The production processes were closely monitored with the view of constructing a workable HACCP plan to enhance the safety of *lafun*. To the best of our knowledge, this study is the most extensive long-term investigation of the production chain of *lafun* to evaluate the microbiological safety of the process.

2. Methods

Sampling

Samples for the study were collected from 16 different *lafun* processors in two villages in Ogbomoso, Oyo State, Nigeria. Sampling was carried out twice a month for six months and the samples were obtained from the same source throughout the sampling periods. Samples were taken at different stages of processing and subsequently analysed for microbiological hazards. The samples collected from the fermentation process include water used for the steeping of cassava, fermenting broths for 24, 48 and 72 h, and the dried samples of *lafun* from lot sizes of approximately 200 l and 75 kg respectively. Sterile bottles of about 5 ml capacity were used. A considerable amount of each of the samples was aseptically collected in the sterile bottle and covered.

An ice bag was used to transport the samples collected into the laboratory to keep the samples at low temperature during the period of sampling and the period of analysis. The samples were analysed within 4 h of collection.

Description of the production sites

During the period of this study, attention was paid to the conditions under which the *lafun* were produced. In this way, data were collected on factors such as sanitation, nature of production sites, types of families and other associated matters in the production process.

pH determination

A portable pH meter model 610A (Fisher Scientific, Pittsburgh, PA, USA) was used to take the pH value of the water samples and fermentation broths at the sites. The pH of the final *lafun* product was also determined by suspending 10 g of sample in 90 ml of sterile water.

Microbiological analysis of samples

The samples were serially diluted with sterile distilled water, and 0.2 ml of appropriate dilution was aseptically plated using the pour plate technique for the enumeration and isolation of mould/yeast, mesophilic aerobic bacteria, coliform, staphylococci, *Salmonella* and lactobacilli on potato dextrose agar (PDA), yeast extract agar, MacConkey agar, mannitol salt agar, *Salmonella-Shigella* agar and De Man Rogosa and Sharpe, respectively (Lab M Ltd., Heywood, UK). The media used were prepared according to the manufacturer's instructions. Plates were incubated at 37 °C for 24 h except for PDA plates that were incubated at ambient temperature of 30±2 °C for 48 h, after which the microbial loads were obtained. Thereafter, colonies were purified to obtain pure cultures and then stored on nutrient agar slant at 4 °C until further use. Distinct colonies of bacteria were identified using their colonial morphology and biochemical tests (Cheesbrough, 2006).

Fungi initially isolated from PDA plates were re-cultured on previously prepared Sabouraud dextrose agar (Lab M Ltd.) and incubated at 30 °C for 72 h. After incubation, the organisms were identified macroscopically by critical examination of culture plates for colonial pigmentation, elevation, size and form of fungi, while microscopic examination was based on the conidial heads, phialides, conidiophres and presence or absence of foot cells or rhizoids (Koneman, 1997).

Antimicrobial susceptibility test

The bacterial isolates were screened for susceptibility using a panel of antibiotics on Mueller Hinton agar plates (Lab M Ltd.) by disc diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1997). The bacterial isolates were tested on the following discs impregnated with antibiotics containing: augmentin (25 μg), gentamycin (10 μg), pefloxacin (10 μg), tarivid (30 μg), sparfloxacin (10 μg), streptomycin (30 μg), septrin (30 μg), chloramphenicol (30 μg), ciprofloxacin (10 μg), and amoxacillin (30 μg) were used for Gram-negative isolates. Pefloxacin (10 μg), gentamycin (10 μg), ampiclox (10 μg), zinnacef (20 μg). Amoxacillin (30 μg), rocephine (25 μg), cipofloxacin (10 μg), streptomycin (30 μg), septrin (30 μg) and erythromycin (10 μg) were used for the Gram-positive isolates. The plates were incubated at 37 °C for 48 h, and afterwards, the zones of inhibition were examined and interpreted accordingly (Chortyk *et al.*, 1993) considering the appropriate breakpoints (Andrews, 2005).

Qualitative detection of aflatoxigenic fungi

All the fungal isolates obtained from different stages of *lafun* production from the 16 processors were inoculated on neutral red desiccated coconut agar (NRDCA; formulated in the laboratory) and incubated at 30 °C for 72 h for rapid detection of aflatoxigenic fungi. NRDCA was prepared following the methods of Atanda *et al.* (2011) as follows: 400 g of desiccated coconut was soaked in 2 l of hot (85 °C) distilled water for 30 min, blended aseptically in a warring blender (Kenwood, Havant, UK) for 5 min and filtered through four layers of cheese cloth (locally sourced). 2% agar (Oxoid, Basingstoke, UK) was added to the filtrate, heated to boiling for 10 min, and then cooled to about 50 °C. Neutral red stain to the tune of 0.3% was added to the filtrate. The medium was then sterilised at 121 °C for 15 min, cooled and poured uniformly into sterile dishes while being vigorously stirred with a sterile hockey stick. Aflatoxigenic positive isolates caused a yellow pigmentation on red colour agar at different times of incubation. Un-inoculated plates of NRDCA served as control for the test.

Quantitative determination of aflatoxin using ELISA

Extraction of aflatoxin from the aflatoxigenic fungi grown on NRDCA plates was carried out as reported by Udom *et al.* (2012) but with slight modification by the addition of 100 ml of 70:30 (v/v) methanol/water (extraction solution) into each of the slants containing the fungus to be extracted. The content in each slant was transferred into a sterile jar until all the contents in the slant bottle were completely removed. The jar was sealed and then mixed for 3 min. The mixture was allowed to settle and the top layer was filtered through a Whatman no. 1 filter paper (Cole-Parmer Instrument Company LLC., Vernon Hills, IL, USA) and the filtrate was collected in a sterile jar. AgraQuant® Total Aflatoxin Assay (Romer Labs Singapore Pte Ltd., Jalan Bukit Merah, Singapore) kit that determines the quantitative level for the presence of total aflatoxins (B_1, B_2, G_1 and G_2) was used according to the manufacturer's instructions. Using the optical density values of the standards, a dose-response curve was constructed from which aflatoxin concentrations of samples were obtained by interpolation.

Hazard analysis of the traditional *lafun* production

The preparation of *lafun* was monitored and the possible sources of hazards were identified through visitation to the production points. The production and storage of the end product were observed as well as other parameters such as hygienic condition of the production environment, personal hygiene of the processors and sources of water used for production. All these were done to identify the likely sources of contamination in the production process. A flow chart for the production of *lafun* was made, while the production of *lafun* was carried out in the laboratory following the method used by the processors, but under stringent hygienic conditions, by incorporating the various control measures/good manufacturing practices (GMP) for the identified sources of hazards. *Lafun* produced in the laboratory was then subjected to microbiological analysis to enumerate mesophilic aerobic bacteria, staphylococci, coliforms, lactobacilli, mould/yeast and *Salmonella-Shigella* as earlier described.

Statistical analysis

The mean values of the total viable bacterial counts obtained from the water, fermenting broths and dry *lafun* samples collected from the processors were subjected to analysis of variance (ANOVA) and the Duncan multiple range test to separate the mean using SPSS (version 10; SPSS Inc. Chicago, IL, USA).

3. Results

The descriptive analysis of the production points is as presented in Table 1. The processors who live mostly in their family houses with their family members carry out the production of *lafun* in the same environment where they rear animals like goat, fowl, etc. Waste dumping sites which at the same time served as toilets were found within 50 m radius of the production points. It was discovered that the water used for the processing was obtained from rain, well, stagnant water or flowing river and stored in open mud or rusty metallic container prior to its use for the fermentation process of *lafun*. The fermented *lafun* was usually dried on a polythene layer in open spaces outside their residences or by the road side on highways. The pH of water used in the production of *lafun* by different processors varied from 7.3 to 7.6, with mean value of 7.5. The fermenting broths on day 1 had pH range of 6.1-6.8 (mean = 6.5), that of day 2 being 4.2-5.9 (mean = 5.1), while the day 3 fermenting broths had pH range of 3.9-4.6 (mean = 4.3). The dried *lafun* samples had pH range of 3.6-4.1 (mean = 3.9).

The microbial attributes of the water, fermenting broths and *lafun* samples are as presented in Tables 2-6. The cumulative microbial counts of the water and fermenting broths from each processor ranged from 1.35×10^4 to 1.04×10^5 cfu/ml, while that of the *lafun* samples ranged from 2.21×10^4 to 9.91×10^4 cfu/g. Generally, all the samples were contaminated, but with varying microbial loads from one processor to the other. The total cumulative microbial loads of the samples obtained from the sixteen processors had the count of 4.26×10^5 cfu/ml for the water samples, which peaked at 6.42×10^5 cfu/ml at the end of 72 h of fermentation. However, the *lafun* samples had the highest total cumulative loads of 6.55×10^5 cfu/g. Among

Table 1. Description of the *lafun* production points.

Parameter/Production point	P_1, P_3, P_6, P_8, P_{12} and P_{15}	P_{13}	P_4, P_5, P_7, P_{10}, P_{11} and P_{16}	P_9, P_2 and P_{14}
Type of building	4-8 room mud house	bungalow	bungalow	traditional mud house
Type of family	extended	extended	extended	extended
Rearing of domestic animals	goats, hens	goats, hens	goats, hens	goats, hens
Involvement of neighbours and children in production	yes	yes	yes	yes
Source of water	rain	flowing river	stagnant water	well water
Presence of toilets	no toilet	pit latrine	pit latrine	pit latrine
Level of personal hygiene	fair	fair	fair	fair
Level of environmental sanitation	fair	fair	fair	fair
Fermenting container	plastic jar	clay pot	plastic jar	plastic jar
Mode of drying	road side	on the rock	road side	road side
Any formal training	no	no	no	no
Storage	sack (28-32 °C)	sack (28-32 °C)	sack (28-32 °C)	sack (28-32 °C)

Table 2. The pH and microbial loads (×10^4 cfu/ml) of water samples used for traditionally processed *lafun.*[1]

Processor/Parameter	pH	AC	CC	SC	SSC	MYC	LC	CUC
P_1W_R	7.5	0.14±0.10^{a}	0.37±0.04^{abc}	0.06±0.01^{a}	0.22 ±0.08^{abc}	0.20±0.04^{a}	0.36±0.04^{bcde}	1.35
P_2W_W	7.5	0.04±0.00^{a}	0.55±0.08^{abc}	0.10±0.01^{ab}	0.12±0.04^{abc}	0.41±0.06^{a}	0.46±0.06^{cde}	1.68
P_3W_R	7.5	2.25±0.23^{e}	1.22±0.15^{d}	1.15±0.19^{d}	0.43±0.05^{cdef}	0.54±0.11^{b}	0.60±0.13^{e}	6.19
P_4W_S	7.5	1.98±0.21^{e}	1.37±0.21^{d}	1.13±0.19^{d}	0.84±0.03^{cef}	0.67±0.06^{b}	0.98±0.13^{f}	6.97
P_5W_S	7.4	0.74±0.18^{bcd}	0.27±0.02^{ab}	0.10±0.02^{ab}	0.69±0.07^{gh}	0.21±0.03^{a}	0.00±0.00^{a}	2.01
P_6W_R	7.5	0.45±0.04^{abc}	0.28±0.02^{a}	0.11±0.01^{ab}	0.37±0.05^{bcde}	0.13±0.01^{a}	0.03±0.01^{a}	1.37
P_7W_S	7.5	0.97±0.24^{d}	0.46±0.13^{abc}	0.16±0.03^{a}	0.15±0.01^{ab}	0.17±0.06^{a}	0.14±0.04^{ab}	2.05
P_8W_R	7.5	0.87±0.24^{cd}	0.54±0.12^{abc}	0.01±0.02^{ab}	0.04±0.03^{abcd}	0.02±0.08^{a}	0.13±0.04^{a}	1.61
P_9W_W	7.5	0.34±0.15^{abc}	0.42±0.04^{abc}	0.41±0.06^{cd}	0.53±0.03^{efg}	0.29±0.04^{a}	0.49±0.06^{de}	2.48
$P_{10}W_S$	7.5	0.36±0.02^{ab}	0.39±028^{abc}	0.33±0.05^{abc}	0.43±0.03^{cdef}	0.27±0.17^{a}	0.40±0.06^{acde}	2.13
$P_{11}W_S$	7.5	0.31±0.03^{abc}	0.33±0.00^{abc}	0.32±0.05^{abc}	0.54±0.02^{efg}	0.24±0.04^{a}	0.29±0.06^{abcd}	2.03
$P_{12}W_R$	7.4	0.26±0.04^{ab}	0.29±0.03^{abc}	0.32±0.07^{abc}	0.34±0.03^{abcde}	0.18±0.02^{a}	0.29±0.06^{abcd}	1.68
$P_{13}W_F$	7.5	0.77±0.01^{cd}	0.65±0.16^{bc}	0.48±0.16^{c}	0.80±0.11^{h}	0.26±0.10^{a}	0.26±0.13^{abcd}	3.22
$P_{14}W_W$	7.5	0.71±0.12^{bcd}	0.37±0.06^{abc}	0.27±0.07^{abc}	0.61±0.11^{fgh}	0.23±0.11^{a}	0.21±0.03^{abcd}	2.40
$P_{15}W_R$	7.5	1.11±0.23^{d}	0.68±0.21^{bc}	0.14±0.09^{ab}	0.14±0.02^{a}	0.18±0.07^{a}	0.36±0.18^{bcde}	2.61
$P_{16}W_S$	7.4	1.17±0.26^{d}	0.69±0.24^{c}	0.16±0.04^{c}	0.33±0.15^{abcde}	0.18±0.05^{a}	0.19±0.09^{abce}	2.72

[1] Mean values (average of twelve) followed by the same superscript in the column are not significantly different by Duncan's multiple range test (P≥0.05). $P_{1\text{-}16}$ = processors; W = water sample; W_R = rain; W_f = flowing river; W_W = well water; W_S = stagnant water; AC = aerobic plate count; CC = coliform count; SC = staphylococcal count; SSC = *Salmonella-Shigella* count; MYC = mould/yeast count; LC = lactobacilli count; CUC = cumulative count.

Table 3. The pH and microbial loads (×10^4 cfu/ml) of fermenting broths at 24 h.

Processor/Parameter	pH	AC	CC	SC	SSC	MYC	LC	CUC
P_1W_R	6.6	1.81±0.24^{gh}	1.95±0.24^{cd}	0.34±0.35^{abc}	0.96±0.19^{b}	0.26±0.37^{abcd}	1.34±0.16^{f}	6.67
P_2W_W	6.3	1.71±0.01^{fgh}	2.24±0.12^{d}	0.57±0.06^{bcde}	0.96±0.21^{b}	0.53±0.17^{cdef}	1.44±0.26^{f}	7.45
P_3W_R	6.4	2.11±0.40^{h}	2.71±0.12^{e}	0.79±0.18^{e}	0.14±0.19^{b}	0.44±0.08^{bcdef}	0.52±0.09^{cd}	6.71
P_4W_S	6.3	1.52±0.34^{efg}	1.67±0.23^{c}	0.59±0.11^{cde}	1.08±0.18^{b}	0.33±0.11^{abcde}	0.62±0.17^{d}	5.81
P_5W_S	6.4	1.24±0.12^{def}	0.24±0.07^{a}	0.63±0.11^{de}	0.28±0.10^{a}	0.48±0.12^{cdef}	0.20±0.05^{abc}	3.07
P_6W_R	6.3	1.09±015^{bcde}	0.36±0.06^{a}	0.80±0.17^{e}	0.26±0.06^{a}	0.74±0.18^{f}	0.15±0.08^{ab}	3.40
P_7W_S	5.7	0.64±0.15^{abc}	0.46±0.15^{a}	0.31±0.07^{ab}	0.27±0.02^{a}	0.21±0.11^{abc}	0.10±0.03^{a}	1.99
P_8W_R	6.3	0.78±0.23^{abcd}	0.43±0.09^{a}	0.17±0.05^{a}	0.19±0.03^{a}	0.27±0.07^{abcd}	0.06±0.02^{a}	1.90
P_9W_W	6.4	0.41±0.05^{a}	0.37±0.04^{a}	0.53±0.20^{bcd}	0.42±0.04^{a}	0.36±0.04^{abcde}	0.59±0.03^{d}	2.68
$P_{10}W_S$	6.4	0.29±0.02^{a}	0.33±0.07^{a}	0.34±0.03^{abc}	0.26±0.03^{a}	0.40±0.04^{abcde}	0.36±0.03^{abcd}	1.98
$P_{11}W_S$	6.4	0.32±0.05^{a}	0.27±0.03^{a}	0.46±0.02^{bcd}	0.35±0.36^{a}	0.40±0.07^{abcde}	0.39±0.05^{abcd}	2.19
$P_{12}W_R$	5.8	0.23±0.03^{a}	0.24±0.03^{a}	0.35±0.24^{abc}	0.38±0.22^{a}	0.32±0.04^{abcde}	0.49±0.08^{bcd}	2.01
$P_{13}W_F$	6.9	0.63±0.05^{abc}	0.63±0.16^{a}	0.55±0.01^{bcde}	1.50±0.07^{c}	0.54±0.07^{def}	0.49±0.08^{bcd}	4.34
$P_{14}W_W$	6.4	1.17±0.68^{cde}	1.23±0.17^{b}	0.35±0.14^{abc}	0.07±0.02^{a}	0.62±0.18^{ef}	0.50±0.06^{cd}	3.94
$P_{15}W_R$	6.5	0.57±0.16^{ab}	0.59±0.90^{a}	0.50±0.04^{bcd}	0.24±0.13^{a}	0.16±0.03^{ab}	0.15±0.02^{ab}	2.21
$P_{16}W_S$	6.5	0.55±0.18^{ab}	0.42±0.05^{a}	0.14±0.06^{a}	0.12±0.01^{a}	0.11±0.02^{a}	0.15±0.03^{ab}	1.49

[1] Abbreviations are as stated under Table 2.

the processors, water samples from processor 4 had the highest cumulative microbial load, while the lowest load was obtained with processor 1. Similarly, *lafun* samples from processor 4 had the highest cumulative load, while the lowest load was obtained with processor 11.

The resistance patterns of bacterial isolates to different antibiotics are shown in Table 7. Only two bacterial isolates showed resistance to combination of two antibiotics; namely augmentin, zinnacef, chloramphenicol and streptomycin. These were strains of *S. aureus* and

Table 4. The pH and microbial loads (×10^4 cfu/ml) of fermenting broths at 48 h.

Processor/Parameter	pH	AC	CC	SC	SSC	MYC	LC	CUC
P_1W_R	4.5	0.71 ± 0.08^{abcde}	0.79 ± 0.01^{cd}	0.61 ± 0.04^{b}	0.39 ± 0.04^{bc}	0.27 ± 0.04^{ab}	0.90 ± 0.18^{bc}	3.67
P_2W_W	5.1	1.12 ± 0.14^{e}	1.25 ± 0.06^{d}	0.77 ± 0.08^{b}	0.59 ± 0.07^{c}	0.51 ± 0.06^{de}	1.23 ± 0.11^{c}	5.47
P_3W_R	5.3	1.81 ± 0.27^{f}	2.12 ± 0.31^{e}	1.07 ± 0.18^{c}	1.10 ± 0.19^{d}	0.84 ± 0.03^{f}	1.15 ± 0.19^{c}	8.09
P_4W_S	5.2	1.94 ± 0.37^{f}	2.81 ± 0.48^{f}	1.12 ± 0.20^{c}	0.88 ± 0.14^{d}	1.57 ± 0.07^{g}	2.05 ± 0.31^{c}	10.37
P_5W_S	5.2	0.65 ± 0.03^{abcd}	0.34 ± 0.11^{abc}	0.16 ± 0.04^{a}	0.34 ± 0.11^{abc}	0.27 ± 0.03^{ab}	0.45 ± 0.09^{a}	2.21
P_6W_R	5.2	0.56 ± 0.03^{abcd}	0.35 ± 0.06^{abc}	0.19 ± 0.06^{a}	0.31 ± 0.11^{ab}	0.26 ± 0.05^{ab}	0.45 ± 0.04^{a}	2.12
P_7W_S	5.2	1.02 ± 0.17^{cde}	0.63 ± 0.14^{abc}	0.19 ± 0.03^{a}	0.28 ± 0.04^{ab}	0.34 ± 0.05^{bc}	0.31 ± 0.05^{a}	2.77
P_8W_R	5.2	0.88 ± 0.15^{bcde}	0.77 ± 0.18^{bcd}	0.23 ± 0.11^{a}	0.22 ± 0.06^{ab}	0.44 ± 0.05^{cde}	0.65 ± 0.19^{a}	3.19
P_9W_W	5.2	0.41 ± 0.04^{ab}	0.49 ± 0.04^{abc}	0.35 ± 0.02^{a}	0.38 ± 0.06^{bc}	0.58 ± 0.02^{e}	0.40 ± 0.03^{a}	2.61
$P_{10}W_S$	5.1	0.38 ± 0.07^{ab}	0.34 ± 0.06^{abc}	0.23 ± 0.01^{a}	0.46 ± 0.05^{bc}	0.29 ± 0.02^{abc}	0.46 ± 0.03^{a}	2.16
$P_{11}W_S$	5.2	0.23 ± 0.19^{a}	0.49 ± 0.09^{abc}	0.20 ± 0.04^{a}	0.29 ± 0.03^{ab}	0.54 ± 0.08^{de}	0.31 ± 0.04^{a}	2.06
$P_{12}W_R$	5.2	0.34 ± 0.04^{ab}	0.47 ± 0.07^{abc}	0.31 ± 0.02^{a}	0.41 ± 0.07^{bc}	0.42 ± 0.06^{cd}	0.31 ± 0.03^{a}	2.26
$P_{13}W_F$	5.3	0.44 ± 0.18^{ab}	0.09 ± 0.02^{a}	0.12 ± 0.06^{a}	0.20 ± 0.05^{ab}	0.40 ± 0.04^{bcd}	0.29 ± 0.04^{a}	1.54
$P_{14}W_W$	5.2	0.53 ± 0.16^{abc}	0.20 ± 0.03^{ab}	0.16 ± 0.09^{a}	0.20 ± 0.04^{ab}	0.26 ± 0.03^{ab}	0.31 ± 0.03^{a}	1.66
$P_{15}W_R$	5.2	0.76 ± 0.24^{abcde}	0.61 ± 0.16^{abc}	0.29 ± 0.06^{a}	0.20 ± 0.02^{ab}	0.18 ± 0.02^{a}	0.30 ± 0.04^{a}	2.34
$P_{16}W_S$	5.1	1.08 ± 0.19^{de}	0.77 ± 0.18^{bcd}	0.17 ± 0.03^{a}	0.09 ± 0.03^{a}	0.53 ± 0.02^{de}	1.13 ± 0.23^{c}	3.77

[1] Abbreviations are as stated under Table 2.

Table 5. The pH and microbial loads (×10^4 cfu/ml) of fermenting broths at 72 h.

Processor/Parameter	pH	AC	CC	SC	SSC	MYC	LC	CUC
P_1W_R	4.1	0.84 ± 0.06^{abc}	1.23 ± 0.03^{def}	0.81 ± 0.16^{a}	0.40 ± 0.09^{abc}	1.40 ± 0.10^{ab}	1.32 ± 0.17^{d}	6.00
P_2W_W	4.2	1.17 ± 0.09^{cd}	1.09 ± 0.27^{cde}	0.76 ± 0.06^{a}	0.49 ± 0.05^{abcd}	1.05 ± 0.07^{g}	1.41 ± 0.20^{d}	5.97
P_3W_R	4.2	1.62 ± 0.47^{d}	1.65 ± 0.22^{f}	1.14 ± 0.14^{ab}	1.08 ± 0.16^{e}	1.25 ± 0.01^{hi}	1.14 ± 0.11^{g}	7.88
P_4W_S	4.2	1.61 ± 0.52^{g}	1.53 ± 0.25^{ef}	1.29 ± 0.17^{ab}	0.59 ± 0.10^{bc}	0.99 ± 0.06^{de}	1.29 ± 0.16^{b}	7.30
P_5W_S	4.2	0.48 ± 0.04^{ab}	0.45 ± 0.09^{ab}	0.42 ± 0.02^{a}	0.20 ± 0.04^{a}	0.82 ± 0.06^{de}	0.42 ± 0.07^{abc}	2.79
P_6W_R	4.3	0.45 ± 0.06^{ab}	0.86 ± 0.03^{bcd}	0.47 ± 0.02^{a}	0.40 ± 0.03^{abc}	0.35 ± 0.07^{ab}	0.40 ± 0.08^{abc}	2.93
P_7W_S	4.1	1.00 ± 0.15^{bc}	0.62 ± 0.12^{ab}	0.22 ± 0.07^{a}	0.43 ± 0.14^{abc}	0.55 ± 0.03^{bc}	0.59 ± 0.15^{bc}	3.41
P_8W_R	4.2	1.05 ± 0.15^{bcd}	0.55 ± 0.15^{ab}	0.24 ± 0.07^{a}	0.33 ± 0.11^{abc}	0.37 ± 0.03^{ab}	0.55 ± 0.13^{abc}	3.09
P_9W_W	4.2	0.57 ± 0.04^{ab}	0.57 ± 0.11^{ab}	0.41 ± 0.06^{a}	0.37 ± 0.04^{abc}	0.62 ± 0.05^{cd}	0.44 ± 0.02^{abc}	2.98
$P_{10}W_S$	4.2	0.36 ± 0.03^{a}	0.37 ± 0.03^{ab}	0.42 ± 0.05^{a}	0.33 ± 0.02^{abc}	0.32 ± 0.01^{ab}	0.29 ± 0.02^{a}	2.09
$P_{11}W_S$	4.3	0.44 ± 0.03^{ab}	0.44 ± 0.05^{ab}	0.39 ± 0.02^{a}	0.26 ± 0.02^{ab}	0.33 ± 0.03^{ab}	0.32 ± 0.04^{ab}	2.18
$P_{12}W_R$	4.2	0.29 ± 0.02^{a}	0.27 ± 0.02^{a}	3.06 ± 2.27^{b}	0.36 ± 0.04^{abc}	0.27 ± 0.04^{a}	0.46 ± 0.06^{abc}	4.71
$P_{13}W_F$	4.1	0.58 ± 0.20^{abc}	0.38 ± 0.03^{ab}	0.35 ± 0.02^{a}	0.73 ± 0.18^{d}	0.33 ± 0.08^{ab}	0.25 ± 0.08^{ab}	2.62
$P_{14}W_W$	4.2	0.62 ± 0.20^{abc}	0.32 ± 0.06^{a}	0.56 ± 0.13^{a}	0.33 ± 0.12^{abc}	0.24 ± 0.11^{a}	0.17 ± 0.08^{ab}	2.24
$P_{15}W_R$	4.1	0.99 ± 0.28^{bc}	0.55 ± 0.13^{ab}	0.62 ± 0.18^{a}	0.62 ± 0.16^{cd}	0.66 ± 0.14^{cd}	0.73 ± 0.14^{c}	4.17
$P_{16}W_S$	4.2	1.12 ± 0.19^{cd}	0.72 ± 0.16^{abc}	0.54 ± 0.10^{a}	0.36 ± 0.09^{abc}	0.36 ± 0.04^{ab}	0.75 ± 0.12^{c}	3.85

[1] Abbreviations are as stated under Table 2.

Salmonella Typhimurium. A total of 71 other bacterial isolates showed multiple-drug resistance, with the number of antibiotics ranging from three to ten. These were strains of *S.* Typhimurium, *E. coli*, *S. aureus* and *Lactobacillus* sp. Similarly, out of 41 fungal isolates obtained, 18 species of *Aspergillus* and one species of *Absidia* were resistant to both nystatin and ketoconazole. Only two isolates of *Rhizopus oryzae* were slightly sensitive to nystatin, while 20 other isolates of *R. oryzae* were completely resistant to both antimicrobials.

Among the 41 fungal isolates obtained in this study, only 16 (about 39%) which include the strains of *Aspergillus fumigatus* and *Aspergillus flavus* showed positive

Table 6. The pH and microbial loads (×10^4 cfu/g) of dried *lafun* samples.[1]

Processor/Parameter	pH	AC	CC	SC	SSC	MYC	LC	CUC
P_1W_R	4.1	0.86 ± 0.11^{cd}	0.52 ± 0.07^{a}	0.55 ± 0.10^{a}	0.49 ± 0.06^{c}	0.43 ± 0.03^{abc}	1.03 ± 0.22^{bc}	3.88
P_2W_W	3.9	1.36 ± 0.18^{ef}	0.67 ± 0.08^{ab}	0.73 ± 0.17^{a}	0.78 ± 0.07^{d}	0.34 ± 0.07^{abc}	1.24 ± 0.14^{cd}	5.12
P_3W_R	3.9	1.18 ± 0.27^{def}	2.21 ± 0.30^{c}	1.34 ± 0.30^{d}	1.06 ± 0.20^{e}	1.53 ± 0.16^{g}	1.63 ± 0.23^{d}	8.95
P_4W_S	4.0	1.41 ± 0.17^{f}	2.39 ± 0.36^{c}	1.75 ± 0.41^{b}	0.84 ± 0.15^{d}	1.32 ± 0.09^{g}	2.20 ± 0.34^{e}	9.91
P_5W_S	3.8	0.80 ± 0.09^{bcd}	0.67 ± 0.03^{ab}	0.56 ± 0.03^{a}	0.37 ± 0.04^{de}	0.93 ± 0.19^{ef}	0.58 ± 0.09^{ab}	3.91
P_6W_R	4.1	0.85 ± 0.05^{bcd}	1.00 ± 0.07^{d}	0.37 ± 0.02^{a}	0.26 ± 0.66^{abc}	0.40 ± 0.79^{abc}	0.83 ± 0.13^{abc}	3.71
P_7W_S	4.0	0.85 ± 0.21^{cd}	0.47 ± 0.12^{a}	0.38 ± 0.13^{a}	0.13 ± 0.04^{ab}	0.81 ± 0.13^{def}	0.62 ± 0.15^{abc}	3.26
P_8W_R	4.0	1.98 ± 0.16^{de}	0.57 ± 0.15^{ab}	0.30 ± 0.15^{a}	0.13 ± 0.03^{ab}	0.63 ± 0.07^{cdef}	0.78 ± 0.18^{abc}	3.39
P_9W_W	4.1	0.45 ± 0.04^{ab}	0.57 ± 0.05^{ab}	0.42 ± 0.03^{a}	0.37 ± 0.05^{bc}	0.88 ± 0.16^{def}	0.50 ± 0.06^{a}	3.19
$P_{10}W_S$	3.9	0.35 ± 0.06^{a}	0.58 ± 0.08^{ab}	0.37 ± 0.02^{a}	0.30 ± 0.05^{abc}	0.48 ± 0.06^{abc}	0.33 ± 0.05^{a}	2.41
$P_{11}W_S$	4.0	0.37 ± 0.05^{a}	0.35 ± 0.04^{a}	0.30 ± 0.08^{a}	0.26 ± 0.05^{abc}	0.54 ± 0.12^{bcd}	0.39 ± 0.03^{a}	2.21
$P_{12}W_R$	4.0	0.47 ± 0.09^{abc}	0.38 ± 0.08^{a}	0.42 ± 0.11^{a}	0.28 ± 0.04^{abc}	0.54 ± 0.09^{bcd}	0.60 ± 0.10^{ab}	2.69
$P_{13}W_F$	4.0	0.50 ± 0.01^{abc}	0.65 ± 0.01^{ab}	0.42 ± 0.04^{a}	0.12 ± 0.01^{ab}	0.16 ± 0.01^{a}	0.41 ± 0.02^{a}	2.26
$P_{14}W_W$	4.0	0.39 ± 0.12^{a}	0.34 ± 0.05^{a}	0.30 ± 0.09^{a}	0.11 ± 0.03^{a}	0.27 ± 0.07^{ab}	1.18 ± 0.03^{cd}	2.59
$P_{15}W_R$	4.1	0.45 ± 0.06^{ab}	0.51 ± 0.10^{a}	0.66 ± 0.08^{a}	0.16 ± 0.02^{ab}	0.57 ± 0.06^{bcde}	0.52 ± 0.06^{a}	2.87
$P_{16}W_S$	3.9	1.00 ± 0.08^{de}	0.56 ± 0.12^{ab}	0.78 ± 0.12^{a}	0.18 ± 0.07^{ab}	0.56 ± 0.06^{bcde}	1.03 ± 0.22^{bc}	4.11

[1] Abbreviations are as stated under Table 2.

reaction for the production of aflatoxin by showing yellow pigmentation on NRDCA. The quantification of total aflatoxin concentration as produced by species of *Aspergillus* isolated from different stages of *lafun* production is shown in Table 8. Strains of *A. fumigatus* produced aflatoxin in different concentrations ranging from 1 to 13 μg/kg, while three strains of *A. flavus* had the highest aflatoxin concentrations of 17, 180 and 1,600 μg/kg.

There are many hazards that are associated with processing steps of traditionally prepared *lafun* as shown in Table 9. Such hazards include chemical hazards in the form of residual pesticides, while the microbial hazards can be encountered in the form of vegetative pathogens from the soil, use of unwholesome water for washing and steeping, and those arising from poor personal hygiene. Sun-drying is another stage in which stones and microbial hazards can be encountered due to contaminated surface in which the *lafun* mash was spread when drying. Container used by the handler for the storage of dried *lafun* such as polythene bags or sacks is also an important source of hazard.

4. Discussion

The analysis of the production points is a pointer to the environmental condition of the processing areas. Results had shown that contamination of *lafun* could have come from the unhygienic environment, probably the presence

Table 7. Resistance patterns of some of the bacterial isolates.

No. of antibiotics	Resistance pattern[1]	Sources of isolates[2]	Isolates	%
2	AU Z	$P_{12}W_R$	*Staphylococcus aureus*	2.81
	CH SXT	P_9W_W	*Salmonella* Typhimurium	
3	PEF AM AU	P_5W_s	*S.* Typhimurium	5.63
	PEF OFX S	$P_{16}W_s$	*S.* Typhimurium	
	PEF APX R	P_3W_R	*Escherichia coli*	
	PEF APX GN	$P_{16}D_2W_S$	*S. aureus*	
4	AU CPX SP SXT	P_8W_R	*S. aureus*	5.63
	E S PEF SXT	P_6W_R	*E. coli*	
	CH CPX SP SXT	$P_{10}W_S$	*S.* Typhimurium	
	AM AU GPX GN	$P_{11}W_S$	*S.* Typhimurium	

Table 7. Continued.

No. of antibiotics	Resistance pattern[1]	Sources of isolates[2]	Isolates	%
5	AM GN PEF R SXT	$P_8D_1W_R$	*Lactobacillus* sp.	14.08
	AM AU E S SXT	$P_{11}D_1W_S$	*S. aureus*	
	AM APX GN PEF R	$P_6D_2W_R$	*S. aureus*	
	APX CPX E GN SXT	$P_7D_DW_S$	*S. aureus*	
	APX GN CPX E PEF	$P_{10}D_DW_S$	*S. aureus*	
	E GN PEF R SXT	$P_5D_DW_S$	*S. aureus*	
	APX GN CPX E PEF	$P_3D_DW_R$	*E. coli*	
	AM APX GN PEF R	$P_8D_DW_R$, $P_1D_2W_R$, $P_5D_3W_S$	*E. coli*	
6	AM APX CH GN PEF R	P_1W_R	*E. coli*	25.35
	APX E GN S SXT Z	$P_9D_2W_W$	*E. coli*	
	AM E S SXT R PEF	$P_3D_2W_R$	*E. coli*	
	APX E GN S SXT Z	$P_{15}W_R$	*S. aureus*	
	APX GN R SXT PEF Z	$P_3D_1W_R$	*S. aureus*	
	AM APX GN OFX PEF R	$P_1D_1W_R$	*S. aureus*	
	CPX E PEF S SXT OFX	$P_{15}D_DW_R$	*S. aureus*	
	AM CH CPX S SXT SP	$P_{14}D_1W_W$	*S. aureus*	
	CPX E PEF S SXT OFX	$P_4D_DW_S$	*S. aureus*	
	AM CH CPX S SXT SP	$P_9D_DW_S$	*Lactobacillus* sp.	
	APX CH CPX PEF SXT OFX	P_5W_S	*Lactobacillus* sp.	
	AM E GN PEF S SXT	P_7W_S	*Lactobacillus* sp.	
	AM AU CPX GN PEF OFX	$P_{12}D_1W_R$	*Lactobacillus* sp.	
	AM AU E GN PEF SXT	$P_2D_DW_R$, $P_{14}W_W$	*Lactobacillus* sp.	
		$P_7D_1W_S$, $P_5D_2W_S$, $P_8D_3W_R$	*S.* typhimurium	
7	APX E GN PEF S STX Z	$P_{13}W_F$	*E. coli*	12.68
	AM APX CPX GN PEF S R	$P_{11}D_3W_S$	*E. coli*	
	AM CH CPX OFX S SP SXT	$P_1D_DW_R$	*E. coli*	
	APX CH GN OFX PEF SP SXT	$P_6D_1W_R$, $P_2D_1W_W$	*Lactobacillus* sp.	
	CPX E PEF S SXT OFX Z	$P_{15}D_2W_R$	*Lactobacillus* sp.	
	CPX E PEF OFX S SXT Z	$P_{12}D_2W_R$	*Lactobacillus* sp.	
	AM CH CPX OFX PEF S SXT	$P_{12}D_2W_R$	*S.* Typhimurium	
	AM AU E GN PEF S SXT	$P_{10}D_1W_R$	*S.* Typhimurium	
8	AM AU CH CPX GN S SP SXT	$P_{15}D_2W_R$	*S.* Typhimurium	9.86
	AM AU CH CPX SP S PEF SXT	$P_5D_2W_S$	*S.* Typhimurium	
	AM AU CH CPX OFX SP PEF SXT	$P_4D_1W_S$	*S.* Typhimurium	
	AM APX GN OFX PEF R S SXT	$P_{16}D_1W_S$	*S.* Typhimurium	
	AM APX GN OFX PEF R S Z	$P_9D_1W_W$	*S.* Typhimurium	
	CPX E PEF S OFX SP SXT Z	$P_{13}D_2W_F$	*Lactobacillus* sp.	
	AM APX CPX GN PEF R S SXT	$P_8D_2W_R$	*E. coli*	
9	AM AU CH CPX E GN PEF OFX R	P_2W_W	*E. coli*	11.27
	AM APX CPX E GN PEF R S SXT	$P_{14}D_DW_W$, $P_{15}D_3W_R$, $P_{13}D_2W_F$, $P_4D_3W_S$	*E. coli*	
	AM APX CPX E GN PEF S SXT Z	$P_7D_3W_S$, $P_{10}D_3W_S$	*S. aureus*	
	AM APX CPX E GN PEF R S Z	$P_{13}D_DW_S$	*S. aureus*	
10	AM AU CH CPX GN PEF R SP SXT Z	$P_7D_2W_S$, $P_{10}D_2W_S$	*S.* Typhimurium	15.49
	AM AU CH CPX GN PEF S SP OFX SXT	$P_{16}D_3W_S$, $P_{11}D_DW_S$	*S.* Typhimurium	
	AM APX CPX E GN PEF R S SXT Z	$P_{16}D_DW_S$, $P_{11}D_2W_S$	*S. aureus*	
	AM APX CPX CH E GN PEF R S SXT	$P_2D_2W_W$, $P_{14}D_2W_W$, $P_6D_3W_R$, $P_9D_3W_W$	*Lactobacillus* sp.	
		$P_{14}D_3W_W$	*Lactobacillus* sp.	

[1] AM = amoxacillin; APX = ampiclox; AU = augmentin; CH = chloramphenicol; CPX = ciprofloxacin; E = erythromycin; GN = gentamycin; OFX = tarivid; PEF = pefloxacin; R = rocephine; S = streptomycin; SP = sparfloxacin; SXT = septrin; Z = zinnacef.

[2] Designation of samples are as stated in Table 2.

Table 8. Total aflatoxin production by the fungal isolates.

S/N	Isolate	Source[1]	Concentration (µg/kg)
1	*Aspergillus fumigatus*	$P_3D_1W_R$	13
2	*A. fumigatus*	P_4W_S	5
3	*A. fumigatus*	$P_2D_DW_W$	1
4	*A. fumigatus*	$P_1D_2W_W{}^2$	9
5	*Aspergillus flavus*	$P_2D_1W_W$	180
6	*A. fumigatus*	$P_3D_DW_R$	4
7	*A. fumigatus*	$P_5D_1W_S$	8
8	*A. flavus*	$P_4W_S{}^2$	17
9	*A. fumigatus*	$P_4D_1W_S$	4.4
10	*A. fumigatus*	$P_{12}D_2W_R$	8.5
11	*A. fumigatus*	$P_3D_3W_R$	6
12	*A. flavus*	$P_2D_DW_W$	1,600
13	*A. fumigatus*	P_1W_R	8.5
14	*A. flavus*	$P_7D_3W_S$	4
15	*A. fumigatus*	$P_4D_DW_S$	3
16	*A. fumigatus*	$P_{13}D_2W_F$	2

[1] $P_{1\text{-}16}$ = processors; $D_{1\text{-}3}$ = day 1-3 samples; D_D = dry *lafun* sample; W_R = rain; W_F = flowing river; W_W = well water; W_S = stagnant water.

[2] Isolate obtained during rainy season.

of animals in the production environment, and water used for processing. Since the water used for the preparation came from natural sources and cassava tubers were covered with soil from farm, contamination may be unavoidable. Several authors have reported high level of contamination of water in the country (Ehiri *et al.*, 2001; Fawole *et al.*, 2002; Lateef *et al.*, 2005, 2012). Contamination may also be from the personnel involved in the food processing during handling, most especially in this case where level of personal hygiene was abysmally low. The acidic pH value of the fermenting broths was not unexpected since cassava is known to undergo acid fermentation during the steeping stage of *lafun* and *fufu* production (Oyewole and Odunfa, 1988; Oyewole and Ogundele, 2001).

The microbial loads obtained in this study fall within the range reported in previous similar studies (Adebayo-Oyetoro *et al.*, 2013; Ijabadeniyi, 2007; Obadina *et al.*, 2009; Padonou *et al.*, 2009). The microbial loads vary widely among the processors, a reflection of quality of water used and hygienic practices. The total microbial counts for all the processors taken together increased within 24 h, with marginal reduction at 48 h before increasing to 6. 42×10^5 cfu/ml at 72 h. The increase in the loads of *lafun* samples may be attributed to post fermentation

Table 9. HACCP control chart for *lafun* production.

Process step	Hazard	Control measure	Critical limits	Monitoring procedure	Corrective action
Steeping	Growth and contamination by pathogenic and spoilage organisms (*Salmonella*, *Escherichia coli*, *Bacillus cereus*, *Staphylococcus aureus*)	Use of potable water, pH control, timing of steeping, cleaning of containers	Potable water; pH 4.7, Fermentation for 84-96 h	pH strips, timing of steeping period, inspection of containers	Rejection of diseased-tuber
Fermentation	Contamination by spoilage microorganisms (*Salmonella*, *E. coli*, *S. aureus*, *B. cereus*, *Aspergillus*)	pH control, proper timing. Proper cleaning of containers and covers	Clean containers, pH 3.6-3.7 84 h	pH strips, appropriate timing	Prolong fermentation time to obtain desired pH
Drying	Contamination by air-borne pathogens and fungi	Use of modern dryer and proper cleaning of the equipment (dryer)	Drying at temperature of 140-180 °C using hot air generated in flash dryer	Thermometer to monitor temperature; ensure product has low moisture content (<14%)	Prolonged drying to ensure moisture content of at most 12%; re-drying to achieve acceptable moisture content
Storage	Growth and contamination by pathogenic and spoilage organisms (aspergilli, bacteria), mycotoxins	Use of neat polythene bag; sealing of the polythene bag; storage at low temperature and humidity; fumigation of store	Storage at low humidity and temperature (not more than 60% and 30 °C)	Visual inspection for change in colour and mould growth, ELISA and UV-light for mycotoxin detection; culturing of samples at regular intervals of storage	Rejection of product with mycotoxin level >15 µg/kg or presence of coliform or any microorganism that may present danger to health of consumers

contamination, since drying was done under open condition. There were occurrences of *S. aureus*, *E. coli*, *S.* Typhimurium, *Lactobacillus* sp., *B. cereus*, *Klebsiella oxytoca*, *A. fumigatus*, *A. flavus*, *Aspergillus niger*, *Absidia corymbifera* and *R. oryzae* in the samples. The presence of wide variety of microorganisms in the *lafun* samples may be a reflection of the unsanitary traditional method of preparation, and drying of fermented cassava mash on road side and subsequent contamination of the flour. *Bacillus* sp., *Klebsiella* sp., *Lactobacillus* sp., *Corynebacterium* sp., *Candida* sp., *Saccharomyces* spp. and *Aspergillus* spp. had been reported as predominant microflora of fermenting cassava for the production *fufu* and *lafun* (Achi and Akomas, 2006; Adebayo-Oyetoro *et al.*, 2013; Oyewole and Odunfa, 1988). The occurrence of these organisms, particularly in the finished *lafun* may portend risks to the consumers. Although *lafun* will be boiled before consumption, some of the organisms found associated with the flour might have produced toxins in to the product before consumption, thereby causing food-borne illness among the consumers.

Antimicrobial drug resistance shown by all the microorganisms isolated from this study is a worrisome development. This in view of potential pathogenicity of some of the isolates and the concern for public health, since major epidemics throughout the world have been reported to be increasingly associated with resistant pathogens (Canton *et al.*, 2003; Levy, 2006). The ability of the microorganisms isolated from this work to withstand the effects of different antimicrobials used could be due to the indiscriminate use of these agents in the environment where *lafun* production was carried out (Malik and Ahmad, 1994; Silva and Hoffer, 1993; Umoh *et al.*, 1990). The processors or other people in the environment could have contributed to the resistance of the bacterial and fungal isolates by use of these agents for both therapeutic and non-therapeutic purposes (Levy and Marshall, 2004). The result of this work also showed that these processors rear all kinds of animals in the environment in which *lafun* production was carried out. Faecal contamination from these animals could have occurred. In addition, it has been estimated that greater than 70% of antimicrobials used are given to food animals (e.g. chickens, pigs, goats and cattle) in the absence of disease (Garofalo *et al.*, 2007), thereby increasing the burden of resistance phenomenon. In our previous works on diverse samples from Nigeria, we have reported high incidence of antibiotic resistance in the country (Adewoye and Lateef, 2004; Lateef, 2004; Lateef *et al.*, 2004, 2005, 2006, 2010).

Several authors have documented the incidence of aflatoxin in cassava products. Kaaya and Eboku (2010) reported aflatoxin range of 0 to 4.5 μg/kg in a cassava sample analysis, while Ogiehor *et al.* (2007) reported aflatoxin range of 0.13 to 4.57 μg/kg in market garri. Also, Essono *et al.* (2008) reported aflatoxin level ranging from 5.2 to 14.5 μg/kg in stored cassava chips, while Njumbe *et al.* (2012) reported concentration levels lower than 12 μg/kg in cassava flour. The results obtained in this study showed the capability of the isolates in producing aflatoxins in far higher level than the permissible level of 15 μg/kg set for the occurrence of aflatoxin in foods by the Nigeria's National Agency for Food and Drug Administration and Control. There seems to be a high burden of incidence of aflatoxin in food samples from Nigeria (Ezekiel *et al.*, 2012; Fapohunda *et al.*, 2012; Lateef and Gueguim-Kana, 2014; Rubert *et al.*, 2013) which is a potent threat to public health. Mycotoxigenic fungi have been greatest concern to food and feed safety worldwide (Moretti *et al.*, 2013). In this work, drying of cassava was observed to last as long as two weeks depending upon weather conditions. Such an extended drying periods promotes insect and mould infestation and can lead to discolouration and changes in flavour of food products (Knoth, 1993). Availability of water in *lafun* due to extended drying periods could be responsible for both mould growth and aflatoxin production (Klich, 2007). Therefore, drying of *lafun* on ground and rock surfaces as practiced by these processors exposes *lafun* to contamination with soil, dust, moulds and other foreign matter thereby promoting contamination. The aflatoxin levels established in this work especially the concentration higher than the recommended limit may have deadly consequences if consumed. Aflatoxin was circumstantially associated with death of a 15 year old boy in Uganda after eating a sample of cassava meal which was later found to contain 1,700 μg/kg aflatoxins (Kaaya and Warren, 2005).

HACCP which was applied in this study has been reported as a means to provide a management tool to reduce or eliminate the hazards caused by bacterial and fungal contamination (Scudamore, 2005). It has been applied in cottage industries, such as production of *fufu* (Obadina *et al.*, 2008), *akara Ogbomoso* (Lateef *et al.*, 2010) and recently, in the small-scale production of poultry feeds (Lateef and Gueguim-Kana, 2014). In the present study, the critical control points were identified as steeping, drying and packaging/storage. The corrective actions can be implemented through education of processors on personal hygiene and food safety, as well as GMP. All these can guarantee the safety of *lafun*, as evident in the low incidence of microbes in the laboratory-prepared *lafun*, which was produced following the corrective actions in the HACCP plan (Table 10). Thus, this work has demonstrated the feasibility of implementing HACCP to improve the safety of *lafun*.

5. Conclusions

This study has examined the processing and production of *lafun* among sixteen processors with the view of determining the microbial attributes, and assessment of hazards associated with its production. The presence of

Table 10. The pH and microbial loads (×10^2 cfu/ml or g) of fermenting broths and *lafun* produced in the laboratory.[1]

	Microbial load				
Sample/parameter	**Water**	**24 h**	**48 h**	**72 h**	**Lafun**
Mesophilic aerobic count	–	1	0.1	10	0.1
Coliform count	–	–	–	–	–
Staphylococcal count	–	–	–	–	–
Salmonella-Shigella	–	–	–	–	–
Mold-yeast count	–	–	–	–	0.1
Lactobacilli count	–	0.1	21	52	0.1
Cumulative load	0	1.1	21.1	62	0.3
pH	7.5	6.4	5.0	4.0	4.0

[1] Each value is an average of three readings, and are within the limits of detection of the methods used.

wide variety of microorganisms in water and *lafun* samples analysed has revealed that contamination may occur in the production of *lafun* especially when it is carried out in an unhygienic environment and by handlers or processors with little or no formal training on food safety. Special attention should be given to effective education of the processors on the microbiological hazards associated with the consumption of poorly processed cassava flour (*lafun*) and the critical control points in the processing of *lafun*. There is need for the provision of potable water at the production sites and supply of modern dryers to the processors to prevent open sun-drying of *lafun*. This can go a long way to eradicate or minimise the level of *lafun* contamination that can result in food poisoning and transmission of food-borne diseases.

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