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*Full Length Research Paper*

# Assessment of microbial air contamination of post processed garri on sale in markets

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**This study was designed to investigate the role of airborne microorganisms in the contamination of post processed garri during sale and distribution under tropical market conditions. Freshly processed garri samples were openly displayed in four different markets in Port Harcourt, Nigeria and the changes in microbial burden of the samples were determined over a period of 30 days. Aerosolised bacterial and fungal counts were determined using the index of microbial air contamination (IMA) procedure by placing sedimentation plates at various locations in stalls where garri was displayed in the markets. The bacteria and fungal IMA levels (55 to 85 CFU/dm$^2$/h) obtained for the different markets were higher than accepted limits (50 CFU/dm$^2$/h) for areas prone to microbial contamination. There was a strong positive correlation ($p<0.05$) between the extent of the microbial burden of the displayed garri products and the level of (a) bacterial IMA (0.978) and (b) fungal IMA (0.947) in the different markets suggesting that microbial burden of the displayed product was due to contamination by airborne microorganisms. Increase in bacterial and fungal loads of the displayed samples during the period of study was significant ($p<0.05$) and ranged from 2.78 to 5.62 LogCFUg$^{-1}$ and from nil to 4.74 LogCFUg$^{-1}$, respectively, while during same period, no significant changes ($p>0.05$) in microbial load was obtained in the control samples (in packages). Microbial counts obtained exceeded the recommended maximum acceptable limit (3.0 LogCFUg$^{-1}$) of mesophilic aerobic bacteria in dried food products. Some the bacterial genera (*Bacillus, Salmonella* and *Escherichia*) isolated from the air around stalls were also isolated from the displayed garri samples whereas all the airborne fungal isolates (*Aspergillus niger, Geotrichum candidum, Penicillium notatum, Rhodotorula* spp., *Fusarium* spp., *Mucor* spp. *and Cephalosporium* spp.) were also present in the displayed garri samples. The results of this study demonstrate the importance of air as the main source of microbial contamination of garri in local markets and the need to encourage hygienic packaging of garri by producers and retailers in order to ensure food safety for millions of consumers.**

**Key words:** Garri, microbial burden, air contamination, market, safety.

## INTRODUCTION

Garri is the most popular fermented food product made from cassava (*Manihot esculenta* Crantz) and is widely consumed as processed by millions of people in West Africa where it forms a significant part of their diet (Edem et al., 2001; Kostinek et al., 2005; Oduro et al., 2000; Ogiehor et al., 2007). It is preferred by urban consumers irrespective of ethnicity and socio-economic class as it is a pre-cooked food product with good flavour (Jekayinfa and Olajide, 2007). The dry form of post processed garri as obtained in markets is commonly consumed without further cooking (soaked in water) with sugar, smoked fish, roasted groundnuts, cooked cowpea and coconut, and sometimes with milk and beverages as

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complements. It can also be prepared into a stiff paste called 'Eba' by adding the granules into hot water and stirring to make a paste of varied consistency which can be consumed with local soups or stews of various types by chewing or swallowing in morsels (Asegbeloyin and Onyimonyi, 2007).

Garri processing covers a series of procedures such as peeling, washing, grating and packing into closely knit bags. A heavy object is placed on top of the bag to express some of the juice and the contents of the bag are then left to undergo spontaneous solid state fermentation for several days at ambient temperatures (Huch et al., 2008; Ray and Sivakumar, 2009). Fermentation of the grated tubers helps in product preservation, flavour development, cyanide reduction and changes in functional properties (Akindahunsi et al., 1999). The fermented pulp is then dried to about 10% moisture content by frying at high temperatures which probably results in partial dextrinization of starch (Osho and Dashiell, 2002), destruction of enzymes and micro-organisms and the expulsion of cyanide gas from the product (Asegbeloyin and Onyimonyi, 2007; Harbor and Ogundu, 2009).

The fermentation of cassava to produce garri provides an enormous scope for value addition and preserves this starchy food in a wide diversity of flavours, aromas and textures that enrich the human diet (Ray and Sivakumar, 2009; Steinkraus, 1997), and helps to ensure distribution and storage of the product without the need for refrigeration. However, post-process problems of garri still persist and include loss of microbial stability and spoilage during storage, distribution and marketing. In Nigeria, the sale and distribution of garri in local markets is associated with practices such as display of product in open buckets, bowls and mats at points of sale and the use of bare hands during handling and sales. These unhygienic practices, which may lead to microbial contamination due to deposition of bioaerosols on exposed products, transfer of microbes from dirty hands and utensils and frequent visits by animals and fomites (which may carry infectious agents), can contribute to the post-process problems of this product. Previous reports have revealed high bioload and a vast array of microorganisms in market samples of garri (Agbonlahor et al., 1997; Amadi and Adebola, 2008; Ijabadeniyi, 2007; Ogiehor et al., 2007). The microorganisms isolated from these market samples include: *Bacillus* spp., *Pseudomonas* spp., *Clostridium* spp., *Salmonella* spp., *Klebsiella* spp., *Aspergillus* spp.; *Penicillium* spp.; *Rhizopus* spp., *Fusarium* spp.; *Cladosporium* spp., etc. These microorganisms can cause deterioration in food quality and spoilage (which may lead to economic losses), serious food borne illnesses and may pose a threat to public health. Moreover, the source of these microbial contaminants may also be a portal for contamination by more potent pathogenic microbes which may cause an epidemic considering the popularity of the food product.

Based on the foregoing, this present study was aimed at investigating the role of airborne contaminants in the post process microbial contamination of garri sold in markets. Freshly processed garri product was openly displayed in four different markets in Port Harcourt, Nigeria to determine the changes in the microbial burden of the product with time as a result of ambient air contamination. The microbial burden of air within the markets was ascertained using the standard index of microbial air contamination (IMA) by settle plates which quantifies the microbial flow directly related to the contamination of surfaces coming from microbes that reach critical points by falling on to them.

## MATERIALS AND METHODS

### Sample collection

Freshly processed garri (50 kg) was obtained from local garri processors in Port Harcourt, Nigeria and distributed in three batches to four different markets under aseptic conditions. Batch A samples consisted of openly displayed garri in sterile basins kept in selected food shops for a period of one month without any form of handling. Batch B samples were openly displayed in sterile basins but covered with an Econet SF mesh (hole size, 0.30 mm × 0.75 mm) to screen out insects and fomites. Samples used as control (Batch C) were kept in hermetically sealed high density polyethylene bags to exclude the influence of the ambient environment. Each of the batches in each market consisted of three replicates. The samples were displayed in the different markets for a period of 30 days and minimal interference from humans and animals was ensured through surveillance and storage in covered clean metal containers at night. Every 5-day interval, representative samples (20 g) of each displayed product and control sample in the four markets were aseptically collected and transferred to the laboratory for microbiological analysis. The freshly processed garri was also subjected to microbiological analysis to determine its baseline contamination before exposure in the markets. All samples were processed within fours hours after collection.

### Determination of index of microbial air contamination in markets

The index of microbial air contamination of the markets was measured thrice a week for a period of one month as described by Pasquarella et al. (2000). This was carried out by exposing a standard Petri dish (9 cm in diameter) containing plate count agar (Biotec) or potato dextrose agar (Biotec) (supplemented with 0.1% concentrated lactic acid) to air according to the 1/1/1 scheme (for 1 h, 1 m from the floor and at least 1 m from walls or any physical obstacle). Thereafter, the plates were closed, transferred to the lab and incubated as previously described. After incubation, the colony-forming units were counted and expressed as total bacterial or fungal counts (CFU/$dm^2$/h).

### Microbiological analyses of garri samples

Ten grams of each sample were aseptically weighed into 90 ml of 0.1% (w/v) sterile peptone water in a sterile 500 ml beaker and allowed to stand for 5 min with occasional stirring using a magnetic stirrer. Thereafter, 10-fold serial dilutions of samples were made

and 0.1 ml aliquot of each dilution was plated on nutrient agar (Biotec) for total culturable heterotrophic bacterial (TCHB) counts and potato dextrose agar (Biotec) supplemented with 0.1% concentrated lactic acid for total fungal counts. The plates were incubated at 37°C for 24 h for bacteria and at 25°C for 3 to 5 days for fungi. After incubation, distinct colonies that developed were enumerated and expressed as colony-forming units per gram (CFU/g) of sample.

Representative bacterial colonies obtained after incubation were purified by subculturing on nutrient agar using the streak plate method after which the purity of isolates was determined using the Gram stain. The purified isolates were then characterized and identified using their colonial, morphological and biochemical characteristics as described by Vanderzannt and Splittstoesser (1992) and Cheesbrough (2000) and with reference to the Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). For fungi, a wet mount preparation of the mycelial growth of each isolate was made using lactophenol cotton blue staining technique and observed under the microscope. The identification of the fungi was based on the examination of the conidial heads, philiades, conidiophore and the presence of foot cells or rhizoids (Samson and Reenen-Hoekstra, 1998).

### Statistical analyses

Statistical analyses were performed on means of triplicate determinations using the MINITAB® 14 statistical software (Minitab, Pennsylvania, USA) by using ANOVA procedure, Tukey's mean comparison tests (with $p < 0.05$) and correlation analysis.

## RESULTS AND DISCUSSION

The results on the IMA obtained for the four markets sampled are as presented in Figures 1 and 2. Data obtained showed that the four markets did not meet the acceptable limit of IMA for public places at risk of microbial contamination. Mean bacterial and fungal IMA values obtained for the four markets were above 55 CFU/dm$^2$/h. Five classes of IMA (CFU/dm$^2$/h) have been devised: 0 to 5 very good; 6 to 25 good; 26 to 50 fair; 51 to 75 poor; >76 very poor and each class represents a different increasing level of contamination (Pasquarella et al., 2000). Maximum acceptable values of IMA related to different infection or contamination risks are 5, 25 and 50 CFU/dm$^2$/h, in places at very high, high and medium risk, respectively and counts above 50 CFU/dm$^2$/h are usually considered as quite unsuitable for areas harbouring unpackaged ready-to-eat food items.

Several species belonging to eight bacterial genera and seven fungal genera were isolated from the air in the four markets. The bacterial species isolated include the following: *Bacillus* spp., *Salmonella* spp., *Pseudomonas aeruginosa, Escherichia coli*, *Micrococcus* spp., *Acinetobacter* spp., *Achromobacter* spp. and *Staphylococcus* spp., while the fungal isolates obtained include species belonging to the following genera: *Aspergillus niger, Geotrichum candidum, Penicillium notatum, Rhodotorula* spp., *Fusarium* spp., *Mucor* spp. and *Cephalosporium* spp. Figures 3 and 4 show the frequency of isolation of the microorganisms from the air in different markets studied. Published reports (Abdel Hameed et al., 2009; Ismail et al., 1999; Yassin and Almouqatea, 2010) have shown that most of the isolated bacterial and fungal genera have been previously isolated from outdoor air. Airborne microorganisms originate from different sources such as soil, animals and humans (Fang et al., 2007; Pósfai, 2008) and the activities of men such as sewage treatment plants, animal rendering, fermentation processes, construction works and agricultural activities play a major role in emitting microorganisms into the air (Gillum and Levertin, 2008; Recer et al., 2001). Bacteria and fungi can also adhere to particles of grain dust and be transported through air. Grain dust is generated during the process of farming and secondary processing of grains (sacking, milling, handling of powdered grains, sorting, etc.) in markets and can play a role as an effective infectious aerosol because its organic materials provide essential nutrients for airborne microorganisms adhered to their surfaces (Kim et al., 2009). Despite the apparent weaknesses of the gravitational sampling technique used in this study as opposed to the use of air sampling impellers, it is not surprising that the atmospheric bioloads of these markets were high considering that these markets were in close proximity to grain milling centers, farms, refuse dumps, road construction sites, abattoirs and grain processing centers. The isolation of *E. coli* and *Salmonella* which usually reside in animal intestines from the air may be attributed to the higher presence of sewage and garbage usually characteristic of urban centers and could have also resulted from faecal particulates spreading by the flapping of wings during the slaughter of birds at the chicken processing centres at the markets. Rupturing of the lower intestinal tract during processing of slaughtered animals at the abattoirs may also be implicated (Lues et al., 2007).

Data on the microbiological analyses of the openly displayed garri samples obtained from the market stalls investigated are as presented in Figures 1 to 6. Bacterial species belonging to 5 genera were isolated and they include; *Bacillus* spp., *Salmonella* spp., *Staphylococcus* spp., *P. aeruginosa and E. coli*. Only *Bacillus* spp., *Salmonella* spp. and *Escherichia* spp. were isolated from both air and garri samples. However, all the fungal isolates obtained from the air (*A. niger, G. candidum, P. notatum, Rhodotorula* spp., *Fusarium* spp., *Mucor* spp. and *Cephalosporium* spp.) were also isolated from the garri samples. Figures 5 and 6 show the frequency of isolation of these microorganisms from the garri samples in the different markets during the 30-day study period. Enumeration of microbial counts in Batch A and B samples in the different markets during the study period indicated a rapid increase in microbial counts of displayed garri samples with time in contrast to the control samples (Figures 7 and 8). For example, TCHB counts in Batch A and B samples displayed in Choba market increased from 2.93 to 5.07 LogCFUg$^{-1}$ and from

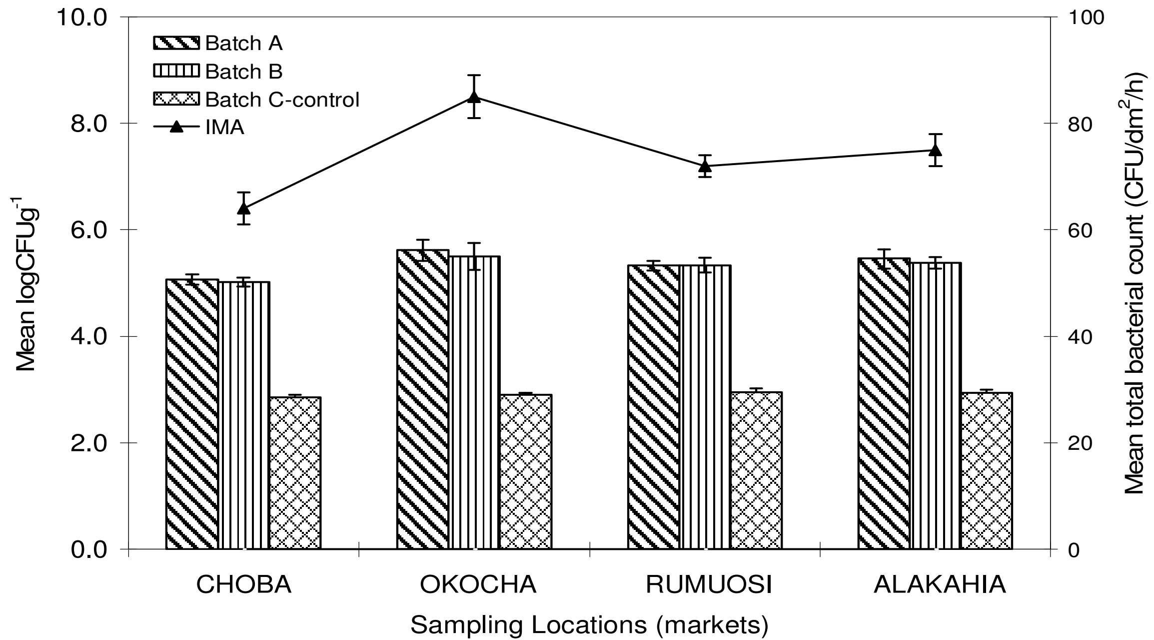



**Figure 1.** Mean total bacterial counts of garri and ambient air obtained from four markets in Port Harcourt. Data represent means of triplicate determinations ± SD.

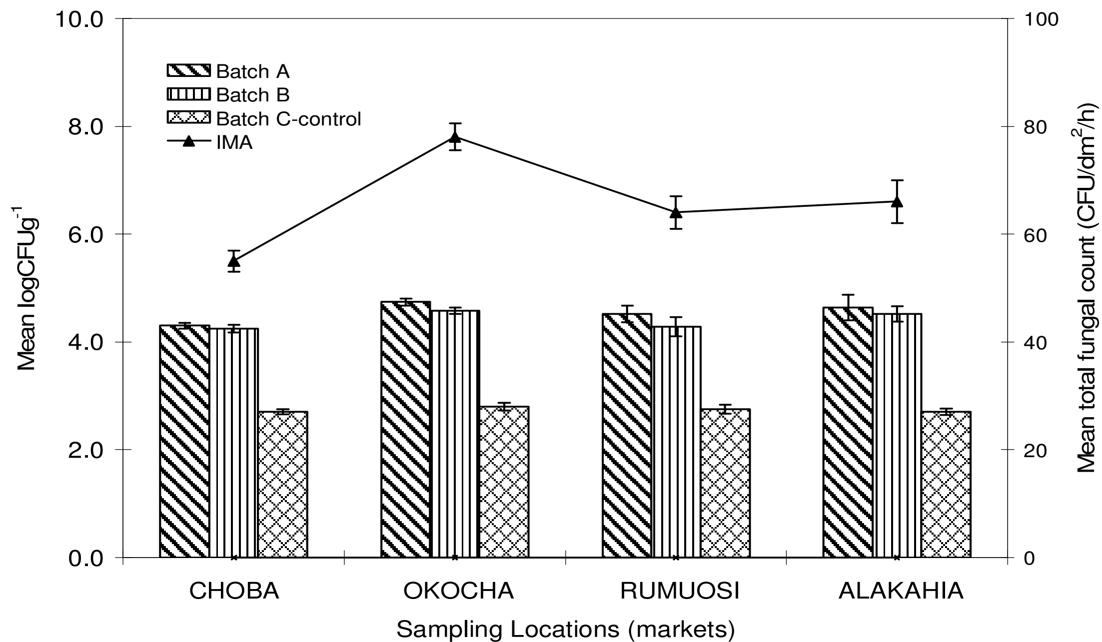



**Figure 2.** Mean total fungal counts of garri and ambient air obtained from four markets in Port Harcourt. Data represent means of triplicate determinations ± SD.

2.84 to 5.02 LogCFUg$^{-1}$ respectively, whereas in control samples kept in the same market, TCHB counts decreased from 2.88 to 2.86 LogCFUg$^{-1}$. Likewise, TF counts for garri displayed in Choba market increased from nil to 4.3 LogCFUg$^{-1}$ and from nil to 4.25 LogCFUg$^{-1}$ for Batch A and B samples respectively with the control samples showing insignificant increase in fungal counts during the period of study. No significant differences ($p>0.05$) were found between mean microbial counts of Batch A and Batch B samples obtained for the different

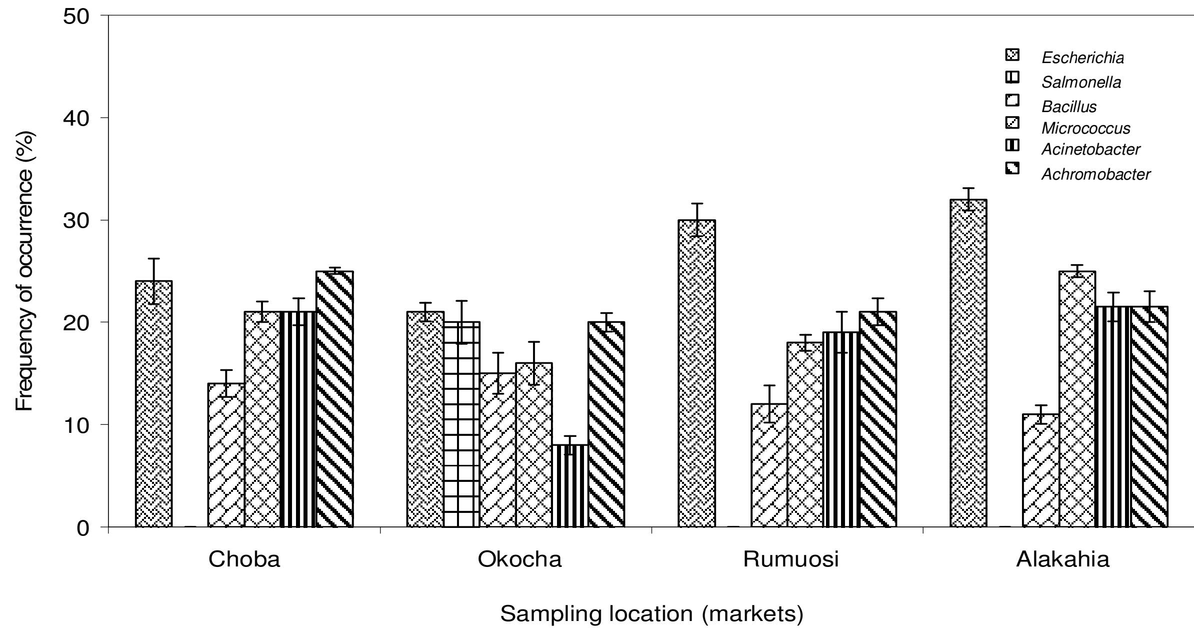



**Figure 3.** Frequency of isolation of bacterial genera obtained from the ambient air in market stalls hosting openly displayed garri samples during the study period. Data represent means of triplicate determinations ± SD.

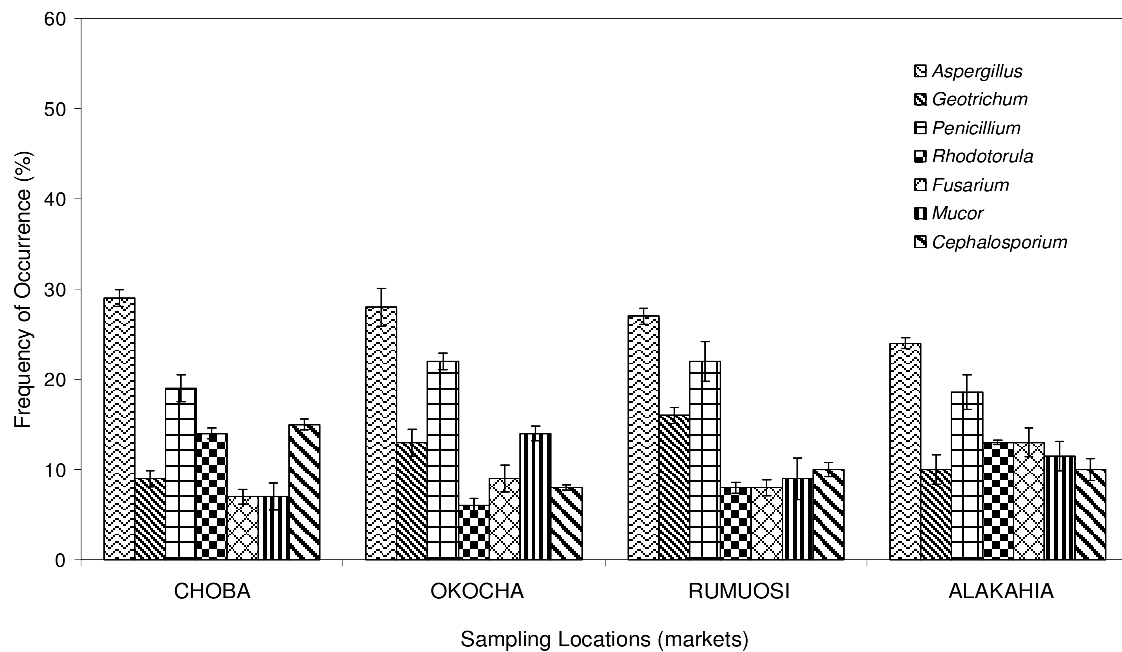



**Figure 4.** Frequency of isolation of fungal genera obtained from the ambient air in market stalls hosting openly displayed garri samples during the study period. Data represent means of triplicate determinations ± SD.

markets. However, there were significant differences ($p<0.05$) between the openly displayed samples (Batches A and B) and the control samples (Batch C). This suggests that visits from insects and other fomites did not significantly affect the microbial loads of the exposed samples. Nonetheless, their role in microbial contamination of ready-to-eat foods cannot be ignored. The mean bacterial counts of the displayed garri samples

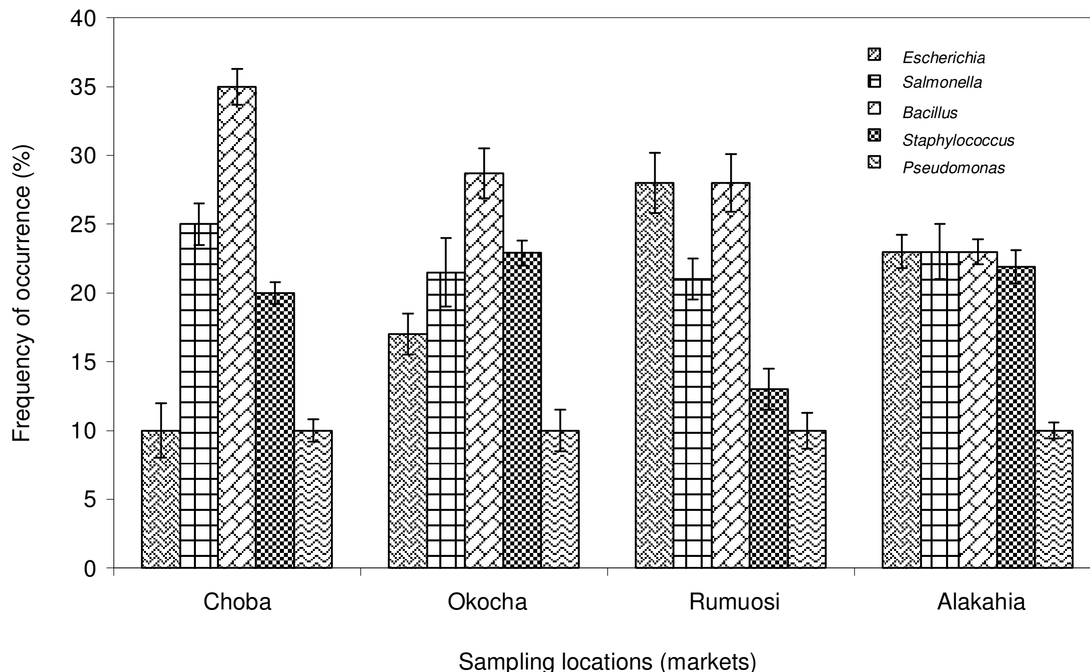



**Figure 5.** Frequency of isolation of bacterial genera obtained from garri samples openly displayed for 30 days in Port Harcourt markets. Data represent means of triplicate determinations ± SD.

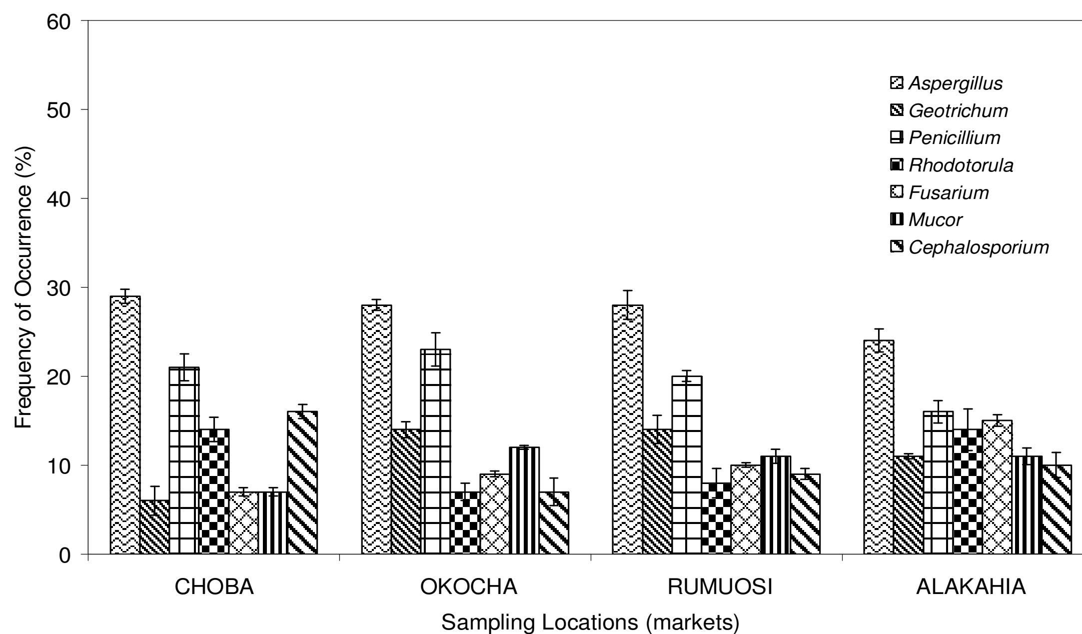
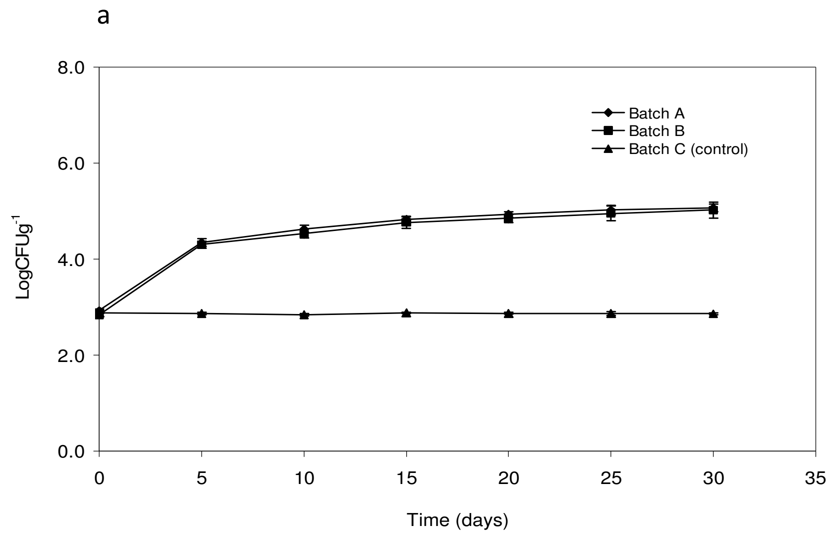
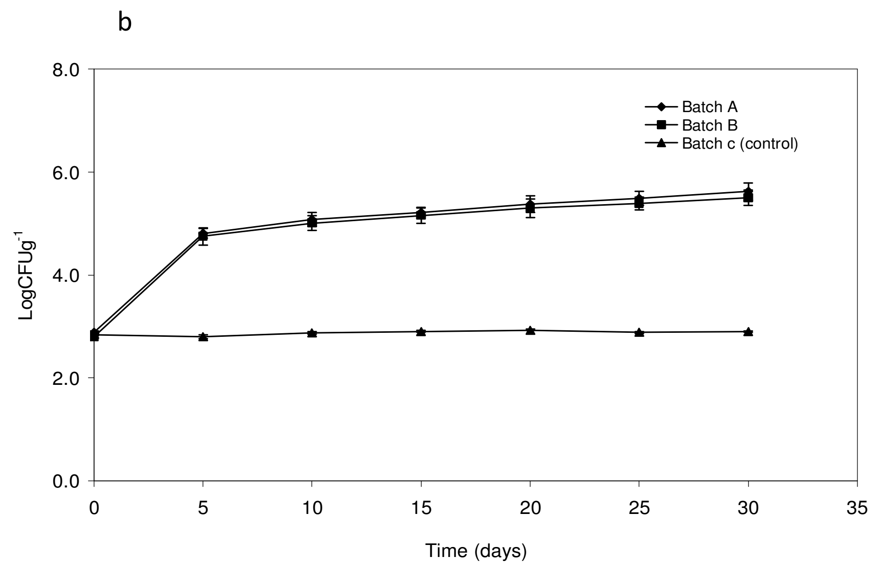



**Figure 6.** Frequency of isolation of fungal genera obtained from garri samples openly displayed for 30 days in Port Harcourt markets. Data represent means of triplicate determinations ± SD.

from the four markets (Figures 1 and 2) obviously exceeded the maximum recommended standards by the international commission on microbiological specification of foods (ICMSF, 1978). According to this agency, the acceptable limit of mesophilic aerobic bacteria in dried food products should not exceed a maximum of 3.0 LogCFU$g^{-1}$.

The extent of the microbial burden of the displayed


a
8.0
6.0
4.0
2.0
0.0
LogCFUg-1
0
5
10
15
20
25
30
35
Time (days)
Batch A
Batch B
Batch C (control)


b
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LogCFUg-1
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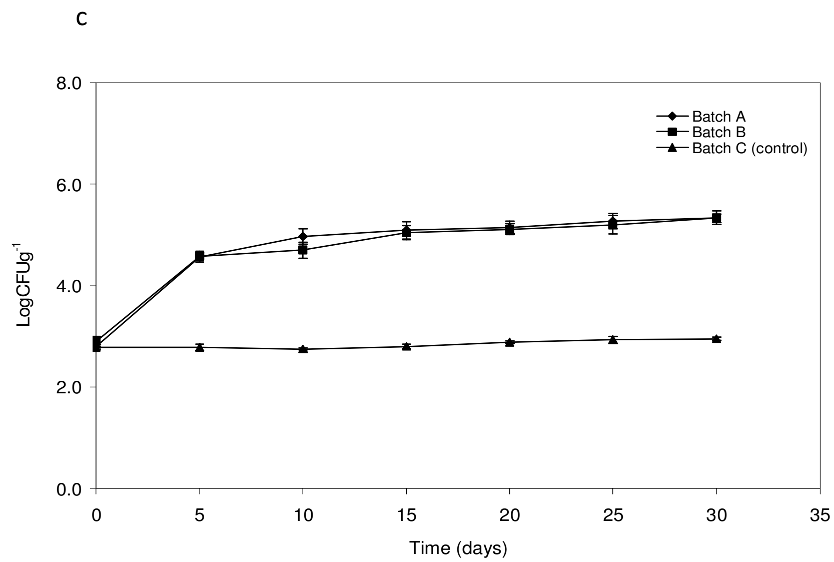
35
Time (days)
Batch A
Batch B
Batch c (control)


c
8.0
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2.0
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LogCFUg-1
0
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Time (days)
Batch A
Batch B
Batch C (control)

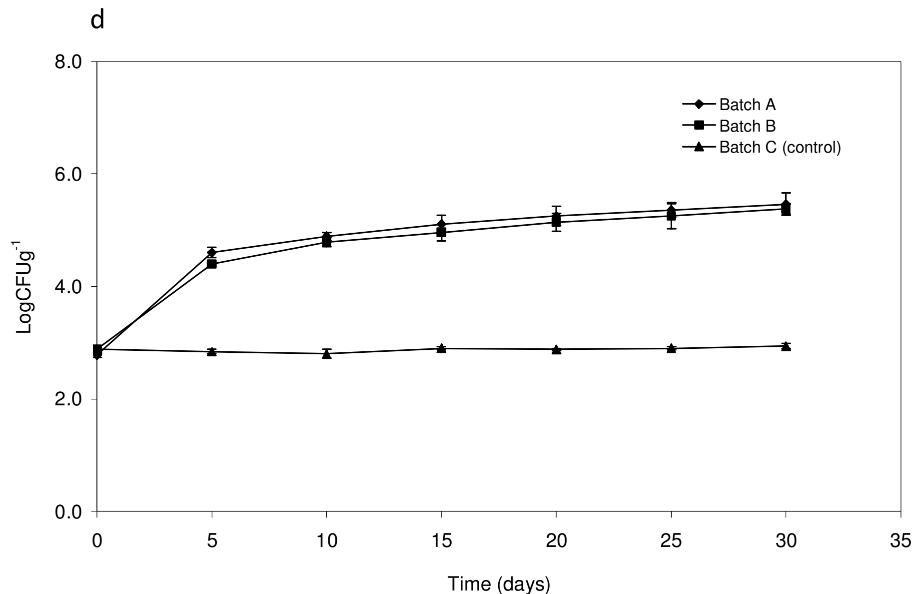


**Figure 7.** Changes in total culturable heterotrophic bacterial counts of garri samples kept in different markets for a period of 30 days. Data represent means of triplicate determinations ± SD. (a) Choba market; (b) Okocha market; (c) Rumuosi market; (d) Alakahia market.

garri products was observed to be dependent on the level of IMA in the different markets. There were strong positive correlations between the mean bacterial counts of Batch A garri samples and the level of bacterial IMA (0.978) and between Batch B samples and the level of bacterial IMA (0.947). Likewise, strong positive correlations between the mean fungal counts of Batch A (0.947) and Batch B (0.857) samples and the fungal IMA were obtained. This suggests that the microbial burden of the air around the market stalls may have been a major factor in the microbial contamination of the displayed samples during the period of study.

Only about 27% of the isolated genera from openly displayed samples (*Bacillus* spp., *A. niger*, *G. candidum*, *P. notatum*) were obtained in control samples. The presence of these microbes in the control samples may be attributed to the survival and resuscitation of their spores after the frying process as they are all known to be spore formers. *E. coli*, *Salmonella* spp. and *Bacillus* spp. were obtained from both air and openly displayed garri samples. In as much as some of the bacterial species isolated from the air were not detected in the garri samples, the bioload obtained in the exposed garri samples may be attributed to the deposition of aerosols on the openly displayed garri. This is because fewer groups of bacteria and fungi were isolated from the control samples shielded from environmental influence after 30 days. Similar observations have been made by Ogiehor and Ikenebomeh (2005) who suggested that processing and handling practices at points of sales may increase microbial contamination.

*Staphylococcus* is found in all individuals and usually expelled from the respiratory tract through the nose and mouth which may also account for their presence in the post processed product. Various researchers (Aboloma, 2008; Oyeyi and Lum-nwi, 2008; Shamsuddeen and Ameh, 2008; Wada-kura et al., 2009) have reported that the presence of *Staphylococcus aureus* in food is an indication of environmental and human contamination. *P. aeruginosa,* an opportunistic pathogen, causes bacteremia and gastrointestinal infections (Kim et al., 2001). *Salmonella* causes food poisoning and typhoid fever (Ekperigin and Nagaraja, 1998; Parry et al., 2002) and are particularly effective at causing human infections because they can survive a series of harsh conditions which include strong acids in the stomach and the anaerobic and salty environment of the intestine that kill most bacteria. *E. coli* may be found in the normal intestinal flora of humans and animals but can also be an important cause of enteric illness and constitute the major etiologic agent of sporadic and epidemic diarrhoea both in children and adults (Nweze, 2010; WHO, 1985). *Bacillus* sp., being a spore former and known to withstand environmental stressors, may have survived the harsh conditions during the processing of the product which may account for its presence in both control and exposed samples. The production of spores enables this organism to withstand unfavourable conditions such as low temperatures or heat and may improve the chances of *Bacillus* to be present in high numbers in the air (Whyte et al., 2001). The most common airborne *Bacillus* was, for many years, dismissed as harmless

contaminants with weak to nonexistent pathogenicity. However, infections are increasingly reported and because the spores are abundant in the environment and usual methods of disinfection and antisepsis are powerless in controlling them, these organisms are becoming a serious health risk (Talaro and Talaro, 1999). Certain strains of *Bacillus* are known causative agents of food poisoning and intoxication and their isolation from garri, which most times requires little or no cooking, is a cause for concern. Several dried food samples have been reported to contain some of these organisms (Blackey and Priest, 1980; Aboloma, 2008). It is noteworthy that some fungal species isolated from garri in the current study have been reported to be involved in human and animal diseases, deterioration of stored products and spoilage of foodstuffs (Burge, 1985; Homer et al., 1994). These fungi have many implications for the spread of human and animal diseases and for the deterioration of stored products.

*Micrococcus* spp., *Acinetobacter* spp. and *Achromobacter* spp. were all isolated from the air around the market stalls but not from the exposed garri samples. Their absence in the garri samples may be attributed to their inability to tolerate the low acid, low moisture and prevailing conditions in the garri. Organic acids (such as lactate, propionate and acetate) produced during fermentation which lower pH of product may be inhibitory to bacteria. Moreover, garri could contain bacteriocins, hydrogen peroxide and other metabolites produced by lactic acid bacteria during fermentation which could be bacteriostatic or bactericidal to certain bacteria. In contrast, the high rate of occurrence and distribution of moulds obtained may be traced to their ability to withstand and tolerate harsh conditions such as the low pH and low moisture content of garri. Previous reports support this observation (Ekundayo, 1984; Ibeh et al., 1991). Tolerance to low pH by *E. coli* and *Salmonella,* facilitated by their inducible acid resistance (AR) systems, may have encouraged their survival despite the environmental stressors prevalent in the fermented product.

In conclusion, results obtained from this study have shown that airborne contaminants in market areas contribute considerably to the microbial burden of garri sold in markets in Port Harcourt. This is exacerbated by the unwholesome but accepted mode of selling and distributing garri in open basins, trays and mats in Nigerian markets. Consequently, the focus on garri should not only be on surface cleaning and hygienic handling during processing, but also on reducing or eliminating microbial air contamination during marketing by packaging of products for sale and distribution as safety is of particular concern with ready-to-eat food products. Adaptations should take the climate and other environmental characteristics of the region into consideration while addressing marketing strategies. The findings suggest that the current mode of sale of garri in local markets may pose potential risks for public health especially for vulnerable people and thus, we recommend the use of hygienic packaging by producers and retailers in Nigeria in order to ensure food safety and consumer protection.

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