

## Full Length Research Paper

# Public health significance of food borne pathogens in edible flours

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Foodborne pathogens cause a considerable public health burden and challenge. They cause illnesses particularly in groups at risk, such as children, elderly and immuno-compromised persons. The microbiological quality of locally produced and industrially processed wheat, unripe plantain, yam and cassava flours were investigated for the presence of foodborne pathogens that could pose a risks to individuals that consume them. Proximate analysis of the flours showed significant statistical differences in the moisture content, crude fibre, lipid, ash, carbohydrate and protein composition of the various flour samples. Bacteria isolated included *Bacillus* sp., *Staphylococcus* sp., *Escherichia* sp., *Salmonella* sp, *Klebsiella* sp, *Enterobacter* sp, *Lactobacillus* sp, *Proteus* sp., *Psuedomonas* sp., *Clostridium* sp. and *Corynebacterium* sp. The frequencies of occurrence of *Staphylococcus* sp., *Escherichia* sp., *Salmonella* sp. and *Klebsiella* sp. in locally produced flours were found to be relatively higher than those isolated from industrially processed and controlled flour samples. Fungi isolated included *Aspergillus flavus*, *A. niger*, *Rhizopus stolonifer*, *Saccharomyces* sp., *Penicillium* sp., *Fusarium* sp, *Mucor* sp, *Candida* sp. and *Geotrichum* sp. In locally produced flours, the frequencies of occurrence of *A. flavus*, *A. niger* and *Rhizopus stolonifer* were relatively higher than those isolated from industrially processed and control samples. Results from this study show that the frequency of occurrence of the potentially harmful organisms such as *Salmonella* sp. and *A. flavus* in locally produced flours exceeds the WHO recommended standards. This may be detrimental to the health of the individuals that consume them. Emphasizing the need for routine medical and laboratory examination of commercially available flour, there should be planning of health education programs for local producers, strict application and implementation of quality control and good manufacturing practice to prevent food borne diseases and ensure the safety of edible flour products.

**Key words:** Flour, food borne pathogens, microbial quality, proximate analysis, food borne illness.

## INTRODUCTION

Flour meals of plantain, wheat, cassava and yam constitute a large part of the daily diet in both rural and urban population of Southern Nigeria. Individuals may

develop diabetes and obesity due to high consumption of foods with a high Glycemic index (Oboh and Erema, 2010). Plantain flour is sometimes used by traditional and

some orthodox medical practitioners in Nigeria in the dietary management of diabetes mellitus and some disease conditions (Eleazu et al., 2011). Unripe plantain meal is usually consumed by Nigerian diabetic patients to reduce post-prandial glucose level (Obboh and Erema, 2010). Unripe plantain flour can be added to other types of flour to produce a highly nutritive product that is beneficial to human diets (Egbebi and Bademosi, 2011).

Wheat flour is a clean, soft and dry whole grain product derived from milling or grinding of fully cleaned moisturized wheat (*Triticum* species) grains; it provides more nourishment for humans than any other food source (Ndife et al., 2011). Whole grains such as wheat are rich in antioxidants which have known health benefits and prevent disease (Slavin, 2004). Semolina is also used to designate coarse middlings from other varieties of wheat, and from other grains such as rice and corn; its consumption is increasing daily among Nigerians because of its smooth fine texture and easy preparation. Whole grains have high concentrations of dietary fiber, resistant starch. Oligosaccharides and epidemiological studies have shown that whole-grain intake prevents cancer, cardiovascular diseases, diabetes, and obesity (Slavin, 2004).

Yam (*Discorea* sp.) is an important source of carbohydrate for many people of the Sub Saharan region, especially in West Africa (Ojokoh and Gabriel, 2010). Yams are processed into dried chips to overcome its high perishability (Hounhouigan et al., 2003). The traditionally processed parboiled dried yam is milled into flour and stirred in boiling water to make a thick paste known as "Amala"; it is eaten with soup by consumers (Akissoe et al., 2001). Yam flour has been fortified with plantain and cassava flour in order to improve its viscosity and texture (Abulude and Ojediran, 2006). Enriching yam flour with soybean can reduce the problem of malnutrition in places where yam is consumed as a staple food (Malomo et al., 2012).

Cassava (*Manihot esculenta* Crantz) is a perennial woody shrub producing edible roots that can be processed into various forms of important food items such as *fufu*, *gari* and *lafun* (Ogiehor et al., 2007; Padonou et al., 2009). *Lafun* is fermented cassava flour, popularly consumed in Southern Nigeria. Cassava shoots contain cyanogenic glycosides that break down to produce hydrogen cyanide, which can cause both acute and chronic toxicity in humans (Ekwu et al., 2005). By adequate processing, cyanogenic glycosides and hydrogen cyanide can be removed or reduced prior to consumption, thus significantly reducing the potential health risk. Continued efforts to improve its nutritional value are important because cassava is a staple food for many people in developing countries (Murtaugh et al., 2003).

The quality of the flour and storage condition after

milling is very important in the shelf life and hygienic quality of the flour. Although flour is generally regarded as a safe product due to its low water activity, a variety of pathogenic and non-pathogenic microorganisms contaminate it during processing (Berghofer et al., 2003). Low-moisture foods and ingredients have not been discussed traditionally in terms of food safety, primarily because these products do not offer welcoming environments for microorganism growth (Akissoe et al., 2001). Yet, flour has also been associated with food borne outbreaks. While most flour-based products undergo a validated kill step at the point of production, such as baking or cooking, many other products may be at risk (Ndife et al., 2011). Improvements in the microbiological safety of foods have been largely driven by public demand in response to disease outbreaks; these improvements have been implemented by international standards and legislation, and are considered to have had an impact on diarrheal incidence as reflected in trends reported worldwide (Nawal et al., 2013).

Diarrheal diseases, almost all of which are caused by foodborne or waterborne microbial pathogens, are leading causes of illness and death in less developed countries, killing an estimated 1.9 million people annually at the global level (Nawal et al., 2013). The presence of aflatoxins in food products such as yam flour, plantain flour, corn flour and others destined for consumption in Nigeria has been reported in previous studies (Ekwu et al., 2005; Ogiehor et al., 2007). This study investigates the microbial quality and proximate chemical composition of unripe plantain, yam, wheat and cassava flours sold for consumption in Port Harcourt metropolis.

## MATERIALS AND METHODS

### Sample collection

A total of 52 flour samples were collected for analysis. Two (2) locally processed yam, unripe plantain, cassava and wheat flour samples were collected for analysis, from each of the following markets: Choba, Oil Mill, Mile I, Mile III and Oyigbo markets in Port Harcourt metropolis. Two samples of different industrially processed flours were purchased at the Everyday Supermarket, Rumuola and labeled appropriately. Dried unripe plantain, wheat, yam and cassava were ground into fine flour, observing HACCP protocols as described by ISO (2005); it was labeled as control. The samples were packaged in polythene bags and transported to the Microbiology Laboratory of the University of Port Harcourt for analysis.

### Proximate analysis

Moisture and ash were determined by the air oven method, crude protein was determined by the micro-Kjeldahl method and the conversion factor from nitrogen to protein was 6.25 (AOAC, 2012).

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**Table 1.** Mean proximate composition of the industrial, local and control samples of different flours.

Flour	Source	Moisture (%)	Lipid (g/100 g)	Ash (g/100 g)	Protein (g/100 g)	Carbohydrate (g/100 g)	Crude fiber (g/100 g)
Wheat	Industrial	29.1 <sup>a</sup>	1.0 <sup>a</sup>	5.6 <sup>a</sup>	7.7 <sup>a</sup>	56.6 <sup>a</sup>	1.8 <sup>a</sup>
	Local	34.7 <sup>b</sup>	1.2 <sup>b</sup>	2.1 <sup>b</sup>	5.9 <sup>b</sup>	53.4 <sup>b</sup>	1.7 <sup>a</sup>
	Control	30.2 <sup>c</sup>	1.2 <sup>b</sup>	2.4 <sup>c</sup>	6.2 <sup>c</sup>	54.3 <sup>b</sup>	1.8 <sup>a</sup>
Unripe Plantain	Industrial	5.7 <sup>a</sup>	2.5 <sup>b</sup>	1.6 <sup>a</sup>	3.5 <sup>a</sup>	82.5 <sup>a</sup>	1.5 <sup>a</sup>
	Local	5.8 <sup>a</sup>	2.6 <sup>b</sup>	1.8 <sup>b</sup>	3.2 <sup>b</sup>	82.6 <sup>a</sup>	1.6 <sup>b</sup>
	Control	5.8 <sup>a</sup>	2.6 <sup>b</sup>	1.9 <sup>b</sup>	3.6 <sup>a</sup>	85.5 <sup>b</sup>	1.7 <sup>b</sup>
Yam	Industrial	9.5 <sup>a</sup>	0.6 <sup>a</sup>	5.2 <sup>a</sup>	7.3 <sup>a</sup>	83.3 <sup>a</sup>	2.3 <sup>a</sup>
	Local	10.3 <sup>a</sup>	0.4 <sup>b</sup>	3.2 <sup>b</sup>	6.2 <sup>b</sup>	87.8 <sup>b</sup>	2.4 <sup>a</sup>
	Control	8.5 <sup>b</sup>	0.5 <sup>b</sup>	4.2 <sup>b</sup>	5.9 <sup>c</sup>	87.9 <sup>b</sup>	2.4 <sup>a</sup>
Cassava	Industrial	15.1 <sup>a</sup>	0.3 <sup>a</sup>	1.4 <sup>a</sup>	1.0 <sup>a</sup>	75.5 <sup>a</sup>	3.6 <sup>a</sup>
	Local	13.8 <sup>b</sup>	0.5 <sup>b</sup>	1.0 <sup>b</sup>	0.9 <sup>a</sup>	77.0 <sup>b</sup>	2.5 <sup>b</sup>
	Control	13.5 <sup>b</sup>	0.6 <sup>b</sup>	1.2 <sup>c</sup>	1.0 <sup>a</sup>	77.2 <sup>b</sup>	2.7 <sup>b</sup>

**Table 2.** Mean heterotrophic bacteria count of the industrial, local and control samples recorded in the different flours.

Source	Plantain (10 <sup>5</sup> CfU/g)		Wheat (10 <sup>5</sup> CfU/g)		Yam (10 <sup>5</sup> CfU/g)		Cassava (10 <sup>5</sup> CfU/g)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Industrial	3.0 - 3.6	3.2	2.3 - 2.6	2.5	3.2 - 4.2	3.6	3.0 - 7.0	4.4
Local	3.1 - 4.9	3.8	3.0 - 4.0	3.4	3.8 - 4.3	4.0	2.5 - 8.6	4.2
Control	3.0	3.0	2.7	2.7	3.2	3.2	3.1	3.1

Crude lipids were determined by the soxhlet extraction method of Egan et al. (1981). Total carbohydrate content was determined by using the Anthrone method (Egan et al., 1981). The crude fiber content was calculated by difference.

#### Enumeration of microorganisms

Enumeration of microorganisms was carried out basically following the approach described by Amoa-Awua and Jakobsen (1995). Enumeration of total viable count was done using plate count agar (Oxoid, CM325, UK). Yeast and mould counts were done on Sabouraud dextrose agar (Oxoid). All cultures were incubated at 37°C for 24 h while yeasts and mould counts were incubated at 25°C for 72 h.

#### Identification of isolates

Pure isolates of the different distinctive bacterial colonies formed were stored on nutrient agar slants at 4°C for further confirmatory tests which included IMVIC test, carbohydrate utilization, and reaction on TSI, gelatin liquefaction, nitrate reduction, urease production and motility. Wet mount of the fungal isolates was prepared in lactophenol cotton blue, examined under low power binocular microscope and compared to the published morphological characteristics of fungi (Watanabe, 2010).

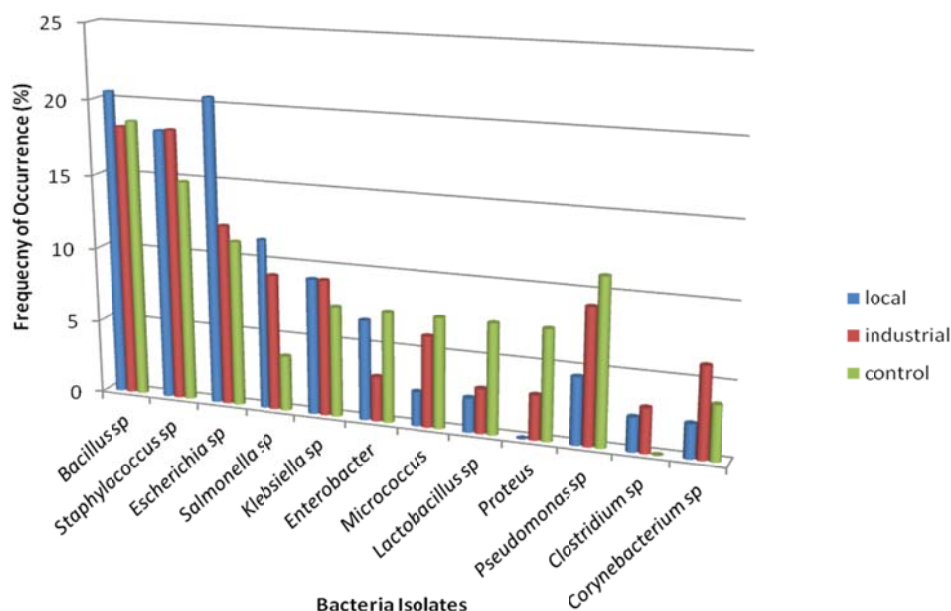
#### Statistical analysis

One way analysis of variance (ANOVA) was used to determine significant differences ( $p < 0.05$ ) within the groups measured at 95% confidence level. The data were entered and analyzed using SPSS (Statistical Packages of Social Sciences) version 16.0.

## RESULTS AND DISCUSSION

The moisture content, crude fiber, lipid, ash, carbohydrate and protein content of the industrial, local and control samples of the various flours are presented in Table 1. Significant statistical differences were recorded in the moisture content, crude fiber, lipid, ash, carbohydrate and protein content of the industrial, local and control samples of the various flours within the groups ( $p < 0.05$ ).

Table 2 shows that the mean heterotrophic count is recorded in the flour samples. Locally produced unripe plantain flour has a mean heterotrophic count of  $3.8 \times 10^5$  CFU/g. Locally produced wheat flour has a heterotrophic count of  $3.4 \times 10^5$  CFU/g; well within the range for safe consumption. Compared to findings of similar studies by Oboh and Erema (2010); Ndife et al. (2011) indicated that wheat flour with heterotrophic count below  $3.5 \times 10^5$  is



**Figure 1.** Comparison of bacteria occurrence isolated from locally produced, industrially processed and control flour samples.

**Table 3.** Mean fungi count of the industrial, local and control samples recorded in the different flours.

Source	Plantain ( $10^3$ CfU/g)		Wheat ( $10^3$ CfU/g)		Yam ( $10^3$ CfU/g)		Cassava ( $10^3$ CfU/g)	
	Range	Mean	Range	Mean	Range	Mean	Range	Mean
Industrial	2.8 - 3.2	3.0	2.3 - 2.6	2.5	2.8 - 3.2	2.7	2.9 - 3.2	3.0
Local	3.1 - 4.9	3.6	3.3 - 3.8	3.5	3.7 - 4.2	3.8	3.4 - 4.2	3.8
Control	3.0	3.0	2.7	2.7	3.2	3.2	3.1	3.1

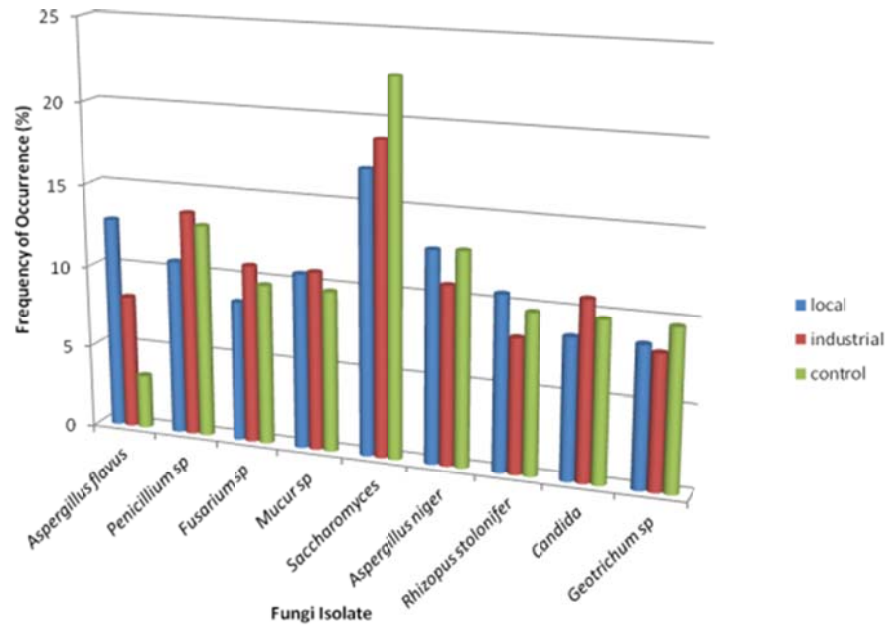
ideal for human consumption and poses little risk of causing food borne illnesses. Heterotrophic counts of locally and industrially processed yam and cassava flours ranged from  $3.6 \times 10^5$  –  $4.4 \times 10^5$  CFU/g. This may be due to the relatively high moisture content of yam and cassava flour (Ojokoh and Gabriel, 2010; Eleazu et al., 2011).

Bacteria isolated included *Bacillus* sp., *Staphylococcus* sp., *Escherichia* sp., *Salmonella* sp., *Klebsiella* sp., *Enterobacter* sp., *Lactobacillus* sp., *Proteus* sp., *Pseudomonas* sp., *Clostridium* sp. and *Corynebacterium* sp. as shown in Figure 1. These bacteria were also isolated from flour samples in similar studies (Ojokoh and Gabriel, 2010; Eleazu et al., 2011). The frequencies of occurrence of *Staphylococcus* sp., *Escherichia* sp., *Salmonella* sp. and *Klebsiella* sp. in locally produced flours were higher than those isolated from industrially processed and control flour samples, except for *Staphylococcus* and *Klebsiella* sp. which had the same frequency of occurrence in locally and industrially produced flours. The presence of these potentially pathogenic bacteria in relatively high frequencies in the

locally produced flour may be attributed to the unhygienic processing environment of locally produced flours (Ogiehor et al., 2007; Nawal et al., 2013). The presence of *Staphylococcus*, *Escherichia* and *Salmonella* in high frequencies is associated with food spoilage, food borne illnesses and food poisoning leading to diarrhea, fever and other health complications in individuals (Adeleke, 2009; Nawal et al., 2013)

Table 3 shows the fungal count of the flour samples. Unripe plantain flour, wheat and cassava flours processed locally had fungal counts ranging from  $3.5 - 3.8 \times 10^3$ . This may be due to the high moisture content of these flours (Abulude and Ojediran, 2006; Ojokoh and Gabriel, 2010). The mean fungal counts of the industrially and locally processed yam flour were  $3.6 \times 10^3$  and  $3.8 \times 10^3$  respectively. This could be attributed to the high carbohydrate and moisture content of the flours (Padonou et al., 2009; Akpe et al., 2010).

Fungi isolated included *A. flavus*, *A. niger*, *R. stolonifer*, *Saccharomyces*, *Penicillium*, *Fusarium*, *Mucor*, *Candida* and *Geotrichum* sp. as shown in Figure 2. These fungi were also isolated from flour samples in similar studies



**Figure 2.** Comparison of fungi occurrence isolated from locally produced, industrially processed and control flour samples.

(Abulude and Ojediran, 2006; Padonou et al., 2009). The frequencies of occurrence of *A. flavus*, *A. niger* and *R. stolonifer* in locally produced flours were higher than those isolated from industrially processed and control samples. Similar studies suggested that high occurrence of *A. flavus* will most likely lead to the high occurrence of aflatoxins in the flour products which will cause complications of food poisoning and related illnesses in the individuals that consume these products (Adeleke, 2009; Gbolagade et al., 2011; Esho et al., 2013)

The relatively low occurrence of potentially harmful bacteria and fungi in industrially processed flours is attributed to the chemical treatments of the flours in a sterile environment during production (Oboh and Erema, 2010; Gbolagade et al., 2011; Esho et al., 2013). This treatment will ultimately reduce the occurrence of these potentially harmful organisms and increase the shelf life of the products.

## Conclusion

The bacterial and fungal counts recorded in most of the flours sampled meet the FAO/WHO, 2012, required microbial limits of not more than  $10^5$  CFU/g. However, the locally made flours had a relatively higher CFU range compared to the industrially made flours. The bacterial and fungal counts recorded in locally made flours were relatively higher compared to the industrially made and control flour samples. This may be due to poor handling during processing and storage. Results from this study show that the occurrence of organisms such as *Aspergillus* sp., *Salmonella* sp. and *Escherichia* sp. was relatively

higher in locally processed flour samples. The locally made flours are readily available and cheaper than industrially processed flours, making them the popular choices among the residents of Port Harcourt. It is therefore important that local producers be educated on the need to observe Hazard analysis and critical control point (HACCP) principles in their environment during production and storage to reduce the rate of occurrence of these potentially pathogenic organisms. Routine inspection of commercially available flours should be carried out by appropriate health institutions to ascertain the quality of these products and ensure their safety for consumption by all individuals.

## Conflict of interests

The authors did not declare any conflict of interest.

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