SRYAHWA PUBLICATIONS

Annals of Microbiology and Infectious Diseases
ISSN: 2637-5346 | Volume 7 Issue 1, 2025
https://doi.org/10.22259/2637-5346.0701002

RESEARCH ARTICLE

Microbial Properties of Wearning Food Flour Prepared From Fermented Maize and Roasted Pigeon Pea Seeds

C.U Okoronkwo, Nebolisah, B.C

Department of MicrobiologyAbia State University, Uturu.

Received : 10 April 2025 Accepted : 25 April 2025 Published: 30 April 2025

Corresponding Author: C.U Okoronkwo, Department of MicrobiologyAbia State University, Uturu.

Abstract

The microbial content of complementary food based on fermented maize and roasted pigeon pea seeds were investigated using standard microbiological identification techniques. The raw maize(RM),fermented maize(FM), raw pigeon pea(RPP),roasted pigeon pea were evaluated alongside the blends of the processed samples to determine the most safest sample for weaning. The results of the microbial load showed that there were significant variations in the microbial loads of the different flours with fermented maize recording highest value ($1.52x10^5$cfu/g). The occurrence of bacteria species in the raw material (flours) used showed 100% occurrence of yeast and *As pergillus sp* in all the four samples, 75% occurrence of *Pennicilliu sp* in three samples (Raw maize, Raw PP and fermented maize) and 25% of *Rhizopus Sp* in Raw PP. The occurrence of fungal isolates showed differences in the levels of occurrence of the four species (mold and yeast) found in the flours. The result of microbial load of the complementary foods based on fermented maize and Roasted pigeon pea flours showed a significant differences ($P<0.05$) in the microbial loads (fungi and bacteria) of the raw materials component used in the fermentation of the food blends (fermented maize and roasted pigeon pea). The occurrence of bacterial isolates in the complementary foods indicated carryover of *microorganisms* in the raw materials (fermented maize and roasted pigeon pea) to the composite blends. *Protus mirablilis* was the least prevalent (40% occurrence) being present only in the fermented maize flour and 50%:50% blends of roasted pigeon pea and fermented maize. The fungal flora of the complementary foods based on fermented maize and roasted pigeon pea flours showed that yeasts and *Aspergillus sp* were present in all food samples (100% occurrence) while *Pennicilium sp* was found in the 60%:40% and 50%:50% (maize-Pigeon pea) samples. It can be concluded that microbial loads could be reduced through heat treatment (Roasting) adopted in this reaserch. The fungal isolates in this work may be laboratory contaminants during processing

Keywords: Maize, Fermentation, Roasting, Pigeon Pea, Microbial.

1. Introduction

Breast milk is the best nourishment for newborns. It contains previously unknown compounds that cannot be replicated artificially, and its total nutritious makeup outperforms any option, even infant formula. Breast feeding is considered best for infants from nutritional and immunological points of view as well as for protection against Campylobacter associated diarrhoea (Nout and Ngoddy, 1997).

Despite its superiority, breast milk cannot provide all of the nutrients and calories necessary for infants to thrive after the first six months and as the child grows, breast milk may no longer satisfy his hunger. Then additional food to the breast milk will be added which is called complementary foods (Okoronkwo *et al*., 2017).

Complementary foods are those foods given to a child in addition to breast milk during the vulnerable

Citation: C.U Okoronkwo, Nebolisah, B.C. Microbial Properties of Wearning Food Flour Prepared From Fermented Maize and Roasted Pigeon Pea Seeds. Annals of Microbiology and Infectious Diseases. 2025; 7(1):18-30.

period of the child (Okoronkwo *et al*., 2017). It can also be refers to those foods that are readily consumed and digested by the young children, and that provide additional nutrition to meet all growing child's needs. Complementary foods (commonly known as weaning foods) are formulated to satisfy nutritional needs of infants and young children. Therefore, the meals must include enough of the necessary nutrients to supplement breast milk. Infants who do not receive enough complementary foods are stunted, malnourished or both as a results of deficiencies in protein, energy and micro-nutrients such as calcium, iron, zinc, vitamin A and iodine (WHO, 2004).

In most developing countries such as Nigeria, Traditionally complementary food is mostly made up of cereal such as maize, sorghum, millet. Maize which is most commonly used is known to be of poor nutritive content combining of low protein, low energy density and high bulk which are all needed for the growth of an infant. For example maize pap has been implicated in the aetiology of protein-energy malnutrition in children during the complementary period (Di Giovanni *et al*., 2005) while legumes seed (pigeon pea) are an important staple food and one of the richest and cheapest source of proteins for majority of people living in developing countries (Aletor and Ojelabi, 2007; Maltas *et al*., 2011; Soris and Mohan, 2011). Therefore, combining cereal with legume would aid to boost the complementary food's nutritional density and increase nutrient intake, which resulted in the prevention of the nutritional issue. Understanding the dietary intakes of infants and toddlers is important because early life nutrition influences future health outcomes (Mannino *et al*., 2004).

Research has shown that processing methods such as fermentation, germination, roasting, boiling, grilling, steaming, etc, have also been shown to have significant effect on the anti-nutritional factors, microbial load, and safety of most traditional complementary foods (Rasane *et al*., 2015). A complementary food developed from maize-pigeon pea mixture using different traditional processing methods would therefore be expected to have certain microbial content which might be harmful or beneficial to infants after consumption. Food safety practice should be put in place in the production of complementary foods hence that it is of high priority for infant growth, preventing mortality and enhancing development (Lutter and Dewey, 2003).

Maize (*Zea mays*) is a perennial grass in the Poaceae family that is used worldwide as a staple food crop. All part of the crop can be used for food and non-food products or consumed as vegetable. It originated from the eastern hemisphere, but is now cultivated in many parts of Africa, North, South, and Central America, Europe and Asia (Leszek and Vincent 2012).

Pigeon pea (*Cajanus cajan* L.), which probably originated from Asia belongs to the family Fabaceae and its cultivation dates back to about 3000 years ago (Eltayeb *et al*., 2010). Pigeon pea, with its protein content of 21-26 % (Oshodi *et al*., 1985; Nwokolo, 1987; Eltayeb *et al*., 2010; Okpala and Okoli, 2011) is highly desirable as a protein supplement to cereal-based diets.

Contamination of complementary foods leads to the occurrence of diarrhoeal diseases in children. This could result from improper processing methods, food handling / handlers and belief system (Sheth and Dwivedi, 2006).

Complementary foods play a crucial role in the growth and development of infants, and it is important to ensure that they are safe and nutritious. *Microorganisms* can affect the safety and nutritional quality of these foods, and it is essential to understand the types and levels of *microorganisms* present in them.

Figure1. *Maize grain Source: https://stock.adobe.com/ng/search?k=maize+grain*

Figure 2. *Pigeon Pea Seed Source: https://www.feedipedia.org/node/329*

2. Materials and Methods

2.1 Source of Materials

The test materials, the raw materials for the formulation of complementary food (maize and pigeon pea seeds) were purchased from Umuahia Central Market, Ubani Ibeku. Laboratory and other facilities were obtained from Ceslab Global Services Analytical Laboratory, Umuahia.

2.2 Preliminary Processing

Both test seeds, yellow maize and green pigeon peas, were separately pretreated prior to their processing into flours used for blending and production of the complementary foods. The following pretreatments were used.

Identification and authentication of the seeds were done by a botanist in the department of Botany, Abia State University, Uturu.

2.3 Production of Fermented Maize Flour

The traditional fermentation method was applied (wet fermentation), the maize seeds were first sorted to remove "bad" ones before a measured quantity (1kg) of the "good" seeds were soaked in clean portable water to cover it very well (about 5 litres). The maize seeds were allowed to ferment for 72 hours (3 days) while the water was changed at 24 hours interval (daily). At the end of the fermentation time (3 days) the seeds were washed in repeated distilled water and spread out to drain on a clean pad on the laboratory bench. After draining dry, the fermented maize seeds were dried in the oven at 50-60^{0}C for six (6) hours before being ground in a laboratory mill to obtain a powdered flour sieved through a 1mm test sieve aperture.

2.4 Production of Roasted Pigeon Pea Flour

The pigeon seeds were first sorted to remove the "bad" ones (Shriveled, infected, rotted discoloured, etc). The seeds were soaked in opions amount of water and the undetected bad ones floated and were scooped out and removed. The good ones were brought out of water and allowed to drain dry before roasting. The process of roasting involved heating the seeds in a dry metal pot with constraint turning to avoid burning. The process was done using gas flame heating which supplied heat at about 120^{0}C and the process lasted for about 10 minutes for 1kg quantity. The roasted seeds were quickly poured out into a clean dry metal tray where it cooled to room temperature. They were examined and burnt ones were carefully removed while the good ones were dehulled by gentle cracking in a laboratory motor with pestle and subsequently winnowed in a tray pan to remove the hulls and collect the hull-free roasted cotyledons which were ground into flour passing 1mn test sieve to give the final product-roasted pigeon pea flour.

2.5 Formulation of Composite Food Flours

The composite complementary food flours were formulated by gravimetric combination of the seed flour as produced above. The combination by weight is shown in the figure below.

Table 1. *Sketch for Formulation of Complementary Food Based on Fermented Maize and Roasted Pigeon Pea*

Produced sample	RM (g)	FM (g)	RPP (g)	PPR (g)
A	100	-	-	-
B	-	25	-	75
C	-	50	-	50
D	-	60	-	40
E	-	70	-	30
R	-	-	100	-

Key: RM = Raw Maize; FM = Fermented Maize; RPP = Raw Pigeon pea; PPR =Roasted pigeon pea

In line with the sketch above, the flours were combined accordingly to form the composite blends. There blends were subjected to analysis as described below.

2.6 Microbiological Analysis

The produced flour blends constituting the complementary foods were analysed for microbiological quality using the following procedures.

2.7 Determination of Microbial Load

Each of the produced food flour was analysed to determine the population (load) of *microorganisms* (bacteria and fungi) using the pour plate technique described by (Chesbrough, 2006). In line with this, the nutrient Agar was used. Each test sample was first diluted serially as shown in the sketch below (Fig. 3).

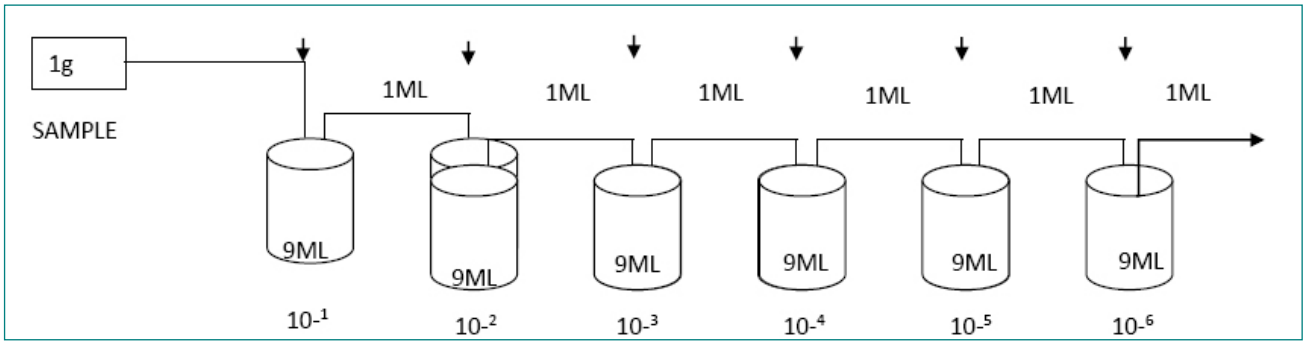

Figure 3. *Sketch for Serial Dilution of Complementary Food Sample for Microbial Analysis*

In accordance with the sketch above, six labelled test tubes were filled with 9mL distilled water each and sterilized by autoclaving and allowed to cool under aseptic condition to room temperature. Exactly 1g of each sample was mixed with 9mL sterile distilled water in a test tube to form the first diluent (10^{-1}). Then from this, 1mL of the diluent was aseptically transferred to the next test tube containing 9mL sterile distilled water to form the second diluent (10^{-2}). This process was continued in series until the sixth test tube (10^{-6}) was reached. The 1mL was aseptically discarded to maintain the 9mL volume in all the tubes. These diluents were used for the work.

The same technique (pour plate) was used for the count of both bacteria and fungi. However, whereas Nutrient Agar was used

for bacterial culture and count, Saborond Dextrose Agar was used for fungi. In this case, exactly 1mL of the 2nd diluent (10^{-2}) and the 4th diluent (10^{-4}) was cultured for bacteria and fungi respectively. The inoculum 1`mL of the appropriate diluent of sample was aseptically collected and dispensed with a clean dry sterile Petridish. However, 15mL of the appropriate medium (NA or SDA) in molten state, was poured aseptically onto the inoculated plate and swirled gently to mix very well.

The plates were allowed to cool and gel at room temperature. The solid inoculated plate were divided according to their medium. The bacterial culture plate (Nutrient Agar) were incubated at 37^0C for 24 hours while the fungal culture plates were incubated at room temperature for 2 – 5 days. In each case, the number of microbial colonies in the incubated plate was counted by means of Gallenkamp electronic colony counter. The formular below was used and the count being the total viable count (TVC), was expressed as the number of colony forming units per gramme of the food (efulg). Counts were taken from plates supporting between 30 and 300 colonies per plate.

$$\text{TVC (cfu/g)} = \frac{1}{v} \times N \times D$$

TVC (cfu/g) = Total Variable Bacteria Count

V = Volume of the diluent cultured

N = Number of colonies counted

D = Dilution factor

2.8 Determination of Microbial Flora

Microbial flora was determined as the different types (species) of bacteria and fungi found in the different food flour samples.

Following the initial growth of *microorganisms* (bacteria on Nutrient Agar and fungi on SDA), the culture plates were examined closely (visually) for distinct colonies from which sub-cultures were made for purification. For the bacteria, a flamed wire loop was used to pick an inoculum from distinct bacterial colonies and streaked onto sterile solid Nutrient Agar plate, labelled and incubated at 37^oC for 24hours. For fungi, a flamed sharp scalpel pomit was used to cut out a piece of the fungal mycelia from a 3 day district colony and placed directly until fresh solid SDA plate.

The inoculum was gently but firmly pressed until the surface of the plate lawn and it was then incubated at room temperature for 2-4 days. The incubated plates were examined for growth in which uniformity of the colonies in each case, marked purity. The pure cultures in each case were used for characterization and subsequent phenotypical identification.

2.9 Characterization of Fungi Isolates

Each of the pure culture of fungi as obtained from the sub-cultures, was subjected to characterization to establish their respective cultural and structural characteristics which were used for matching with existing taxa in standard manual (fungi atlase) for identification.

2.10 Cultural Characteristics

The colonies in a pure fungal culture were observed and examined closely and the features found in the colonies in plate were recorded including the extent of growth, colour, pigmentation, presence of visible mycelia, form of the surface and underneath, presence of fruiting bodies like spores, etc. all isolate.

2.11 Structural Characteristics

To study the structural characteristics of fungal isolate, each isolate was mounted on a slide and stained with lactophenol cotton blue dye solution, the slide mount was viewed under x40 magnification objective lens. Observed features were recorded including to form and directional growth of the mycelia, presence of septates, branching, form of apex growth as well as presence and shape of fruiting bodies like spores and conidia. All observed features were recorded against each isolate for comparison with existing taxa in the manuals.

2.12 Characterization of Bacterial Isolates

Each of the bacteria isolated from the samples (through the sub-cultures) was subjected to characterization.

2.13 Colony Features

Each pure culture of bacterial isolate was examined in plate (visually) and the features were recorded including the extent of growth, colour, colony elevation, pigmentation, form of the edge (Margin), consistency etc. the observed features were recorded accordingly (and later crosschecked with taxa in manuals).

2.14 Microscopic Features

Slide mount were made for each isolate and stained with the principle dyes (gram stain) to classify it as gram positive or negative. Also the shape and arrangement or the cells were also noted. Some future microscopic works were done in which specific stains were used to check the presence (or otherwise) of specific features like 5 pores, flagella etc. All observed features were recorded accordingly.

2.15 Biochemical Reactions

In furtherance of bacterial characterization, each isolate was tested for different biochemical reactions including ability to produce enzymes such as catalase, oxidase, coagulase and urease. Also the ability to reduce nitrate, utilization of citrate, etc.

Also tests were conducted on the ability of the isolates to utilize different sugars like glucose, maltose, sucrose, lactose, mannitol and xylose with possible production of acid or gas or both. All observed features were also recorded accordingly.

2.16 Determination of Coliform

To detect the presence (or otherwise) and the population of coliform in the produced complementary foods, a portion of the food was cultured in MacConkey Agar and observed for the characteristics feature of coliform on the selective medium.

3. Result and Discussion

Table 2. *Microbial Load of Raw materials used in the Production of Complementary Foods*

Sample	THBC (cfu/g)	TFC (cfu/g)
Raw maize	$6.9x10^4$	$8.33x10^2$
Raw PP	$9.17x10^4$	$1.10x10^3$
Fermented Maize	$1.52x10^5$	$1.53x10^3$
Roasted PP	$4.77x10^4$	$4.33x10^3$

Values show means of triplicate analysis. Figures with different superscript in the column are significantly different ($P<0.05$)

THBH = Total Heterotrophic Bacterial Count

TFC = Total Fungal Count

3.1 Microbial Load of Raw Materials

Table 1 show the microbial load of the raw materials used in the production of the complementary foods based on fermented maize and roasted pigeon pea. From the results, there were significant variations in the microbial load of the different flours. The

bacterial load of the fermented maize was higher ($1.52x10^5$cfu/g) than that of the raw seed flour ($6.90x10^4$cfu/g) whereas the bacterial load of the roasted pigeon pea seed flour was much lower ($4.77x10^4$cfu/g) than the raw pigeon pea seed flour ($9.19x10^4$cfu/g). A similar trend was observed in the fungi load (mold and yeasts) of the flour. A higher total fungi count (TFC) of $1.53x10^3$cfu/g was recorded in the fermented maize flour as against $8.33x10^2$cfu/g recorded in the raw maize flour.

The general high microbial load of the fermented maize flour could be attributed to chance inoculation and proliferation of *microorganisms* in the course of the wet fermentation. Also the relative higher microbial load of the raw pigeon pea flour compared with the roasted seed flour could be attributed to the rich nutrient level of the legume which encourages the proliferation (multiplication) of *microorganisms* whereas the roasting temperature tend to kill most microorganism thus the low microbial load recorded. It is also reported that fermentation increases the nutrient level of seeds hence more nutrients available to *microorganisms* which find their ways into fermented foods.

Generally therefore, whereas the fermentation of maize seed led to higher microbial load and flora, the process of roasting significantly reduced microbial load and flora pigeon pea seed flours. This observation pre-supposes that the nitrification (fortification) of maize flour (a cereal) with the more nutrition's pigeon, when roasted, does not pose much problems from the microbiological view point.

Table 3. *Occurrence of Bactria in Raw Materials for the Production of Complementary Food based on fermented Maize and Roasted Pigeon Pea*

Sample	Streptococcus spp	Bacillus spp	Proteus spp	Pseudomonas spp	Lactobacillus	Shptococcus spp
Raw maize	+ve	+ve	-	+ve	+ve	-
Raw PP	+ve	+ve	-	+ve	+ve	-
Fermented maize	+ve	+ve	+ve	+ve	+ve	+ve
Roasted PP	+ve	+ve	-	-	-	-
% Occurrence	100%	100%	25%	75%	50%	25%

Key
+ve =Present(isolated) ; - =Absent(not isolated)

3.2 Occurrence of Bacterial in Raw Materials

Table 3 show the occurrence of bacteria species in the raw material (flours) used for the production of complementary foods based on fermented maize, and roasted pigeon pea. The result show high prevalence of *staphylococcus* and *Bacillus* species. In all the flour samples (100% occurrence). In contrast, *Staphylococcus* species and Proteus had relatively low occurrence of 25% each while *Lactobacillus* and *Pseudomonas* recorded 75% occurrence each.

The presence of *Staphylococcus* species to its Ubiquitous habitation on one hand and also to the fact that it is a natural inhabitant of the human skin and can thus easily contaminate food though the handlers. Also, *Bacillus* species are ubiquitous and in addition, have the ability to form dry spores which can be airborne, carried by wind and settle in any environment. This makes them easy contaminants in food processing operations. The result show a relatively fewer bacteria in the roasted pigeon pea flour and this was attributed to perhaps the effect of heat which may have killed source bacteria leaving only those that can contaminate the flour after production possibly from the laboratory environment.

Notwithstanding, the presence of few bacterial species, it was observed that no coliforms were found in the flours and this gives credence to the sanitation level of the production process. This imply a significantly safe level of the raw materials (fermented) maize and roasted pigeon pea flours) used in the production of the complementary foods.

Table 4. *Occurrence of Fungi in Raw Materials*

Sample	Aspergillus sp	Rhizopus sp	Pennicillium sp	Yeasts
Raw maize	+ve	-	+ve	+ve
Raw PP	+ve	+ve	+ve	+ve
Fermented maize	+ve	-	+ve	+ve
Roasted PP	+ve	-	-	+ve
% Occurrence	100%	25%	75%	100%

Key
+ve =Present(isolated) ; - =Absent(not isolated)

3.3 Occurrence of Fungi in Raw Materials

Table 4 show the occurrence of fungi isolate in the raw materials used in the formulation of the complementary foods. The result show differences in the levels of occurrence of the four species of fungi (mold and yeast) found in the flours.

Yeasts and a *Aspergillus* were presented in all the flours (raw and processed maize and pigeon pea) scoring 100% occurrence while penicillin was found in three of the four flours (75% occurrence) and rhizomes was present in only the fermented maize flour.

The high prevalence of a *Aspergillus* and *yeast* could be attributed to their ubiquity (present in diverse habitant) on one hand and their respective ability to far species which can be airborne and dispersed by wind on various environment. By this, there species can become contaminant of most foods by Rhizomes was only found in the fermented maize. Most Rhizomes are saprophytic and align with decaying materials such as was the case in many fermentation processes such as the case with wet fermentation of the maize seeds, also, the heat of roasting of the pigeon pea may have destroyed the fungi (especially the susceptible ones) leaving the seed flour too dug (low moisture) which discourage the settlement and subsequent growth of fungi spores.

Table 5. *Microbial Load of Complementary Food Based on Fermented Maize and Roasted Pigeon Pea*

Food sample flour	TBHC (cfu/g)	TFC (cfu/g)
100% FMF	$1.5x10^5±7.10$	$1.53x10^3±2.52$
25:75	$3.28x10^4±4.14$	$6.9x10^2±3.61$
50:50	$6.57x10^4±1.16$	$8.61x10^2±0.58$
60:40	$1.09x10^5±3.06$	$1.13x10^3±0.58$
70:30	$1.42x10^5±2.22$	$2.1x10^3±1.16$
100% RPP	$4.47x10^4±1.53$	$4.3x10^2±1.16$

Values show means of triplicate analysis ± standard deviation. Figures having different superscripts in the column are significantly different (P<0.05)

3.4 Microbial Load of Complementary Foods Based on Fermented Maize and Roasted Pigeon Pea

Table 5 show microbial load of the complementary foods based on fermented maize and roasted pigeon pea flours. Significant differences (P<0.05) were observed in the microbial loads (fungi and bacteria) of the raw materials component used in the fermentation of the food blends (fermented maize and roasted pigeon pea) as well as between the composite foods. From the results, mean bacterial counts (loads) of $1.52x10^5$cfu/g and $4.47x10^4$cfu/g were recorded for fermented maize flour and roasted pigeon pea flour respectively. This result show that there were more bacteria cells in the fermented maize than was observed in the roasted pigeon pea. Also fungi load of the fermented maize was higher (1.53cfu/g) than the roasted pigeon pea flour ($4.3x10^2$cfu/g). This generally higher microbial load (bacteria and fungi) in the fermented maize could be due to the process of fermentation wherein there was proliferation and free multiplication of *microorganisms* in the fermenting liquors.

In the produced foods, the microbial load varied significantly with blends. At 25% (W/W) inclusion of maize (i.e. 25:75), the bacterial load was on average of $3.28x10^4$cfu/g but increased to $1.42x10^5$cfu/g in the blend containing 70% maize flour (70:30). In the 60:40 blended flour, the bacterial load was $1.09x10^5$cfu/g while the fungal load was $1.13x10^3$cfu/g. The bacterial load decreased with increase in the roasted pigeon pea flour from $1.07x10^5$cfu/g to $6.57x10^4$cu/g at 50:50 blending. The same trend was observed in the fungal load which decreased from $1.13x10^3$cfu/g in 60:40 blend to $8.61x10^2$cfu/g in the 50:50 blended food.

In general therefore, the microbial load appeared to decrease with more roasted pigeon pea addition and increased with fermented maize flour addition in the complementary foods. Notwithstanding, the overall microbial count does not call for serious concern since most of the foods have bacterial load below the minimum acceptable criteria for foods which is $1.0x10^5$cfu/g. In standard acceptable limit while the 60:40 blend was as the fresh hold. It is also observed that the production process of complementary foods usually involve reconstitution in hot water which in itself will also effect significant reduction in the microbial load of the food before its consumption.

Table 6. *Occurrence of Bacteria Isolates in Complementary Foods Based on Fermented Maize and Roasted Pigeon Pea*

Sample	Streptococcus spp	Bacillus subtilis	Pseudomonas aeruginosa	Proteus mirabils	Lactobacillus spp
Fermented maize flour 100:0	+ve	+ve	+ve	+ve	+ve
25:75	+ve	+ve	+ve	+ve	+ve
50:50	+ve	+ve	+ve	+ve	+ve
60:40	+ve	+ve	+ve	-	+ve
70:30	+ve	+ve	-	-ve	+ve
Roasted pigeon pea 0:100	+ve	+ve	-	-	-
Occurrence	100%	100%	66.67%	50%	83.33%

Key

+ve =Present(isolated) ; - =Absent(not isolated)

3.5 Occurrence of Bacteria in Complementary Foods Based on Fermented Maize and Roasted Pigeon Pea

Table 6 show the occurrence of bacterial isolates in the complementary foods based on fermented maize and roasted pigeon pea. The result is indicative of a carryover of *microorganisms* in the raw materials (fermented maize and roasted pigeon pea) to the compounded foods. From the result, the fermented maize flour labored wee bacterial species than the other flour blends including species of *Staphylococcus*, *Pseudomonas, Bacillus, Lactobacillus* and *Proteous. Proteous morablis* was the best prevalent (40% occurrence) being present only in the fermented maize flour and the 50:50 blend of roasted pigeon pea and fermented maize. *Bacillus* and *Staphylococcus* species were found in all the food blends as well as in the raw materials (100% occurrence). *Lactobacillus* had 80% occurrence being absent only in the roasted pigeon pea flour while *Pseudomonas aeruginosa* was present three samples only (60% occurence).

The occurrence of *Bacillus* and *Staphylococcus* species could be due to contamination from the environment, materials or even handlers in the process of production since both are quite widespread in habitat. *Lactobacillus* may have come from the fermented maize flour since it is among a lactic acid bacteria (LAB) which are generally involved in starch fermentation. On the other hand, *Pseudomonas aeruginosa* and *Proteus mirabilis* are both associated with water and may have originated in the water used during fermentation and washings in the production process. Concerns should be raised in the bacteria flora of the complementary foods especially with the presence of *Staphylococcus* which is associated with pathogenicity and toxicity. However, *Bacillus subtilis* has the ability to produce heat stable spore but the level of pathogenicity of *Bacillus subtilis* is negligible. *Lactobacilli* are generally probiotics and belong to the GRAS organisms (Generally Regarded as safe) and have also found application yoghurt etc.

In the light of the above, it was observed that a little improvement in the sanitary condition of personal and environment of production of their complementary foods will meet up with safety concerns.

Table 7. *Occurrence of Fungi Isolates in Complementary Foods Based on Fermented Maize and Roasted Pigeon Pea*

Food Sample flour	Aspergillus	Yeasts	Pennicilium	Rhizopus spp
FMF	+ve	+ve	+ve	+ve
25:75	+ve	+ve	+ve	-
50:50	+ve	+ve	+ve	-
60:40	+ve	+ve	+ve	-
70:30	+ve	+ve	+ve	-
RPP	+ve	+ve	-	-
% occurrence	100%	100%	83.33%	16.67%

Key

+ve =Present(isolated) ; - =Absent(not isolated)

3.6 Occurrence of Fungi in Complementary Foods Based Fermented Maize and Roasted Pigeon Pea

Table 7 show fungi flora of the complementary foods based on fermented maize and roasted pigeon pea flours. Few fungi species were found in the complementary foods including *Aspergillus* species

and *Penicillin* species as well as yeasts. The yeasts and *Aspergillus* were present in all food samples (100% occurrence) while *Penicilium* was found in the 60:40 and 50:50 (maize-Pigeon pea) samples only. The yeasts were believed to have originated by chance, inoculation into the fermenting maize seeds and later perhaps carried over to the final food products. Like the yeasts also, *Aspergillus* spore fill the wild air and can easily be deposited on to any favourable spot from what it can grow and multiply.

The absence of *Rhizepus, a saprophytic fugues,* in the complementary maize whereas it was in the fermented maize, flour could man it was first transient in the maize flour possible being found by chance at the time of analysis. It could also here been a contaminant from pictorials or personal at the time of analysis. Yeasts are normal constituents of most open and fermented foods and are not associated with pathogenicity.

4. Summary

The study dealt on microbial content of complementary food based on fermented maize and roasted pigeon pea study. Specifically, identified and quantified the types and levels of *microorganisms* present in the fermented maize and roasted pigeon pea based complementary food; determined the effect of the fermentation process on the microbial content of the food, and identified any potential changes in the microbial population over time; evaluated the safety of the complementary food by identifying and quantifying any potential pathogenic *microorganisms* present. (Walker, 1994).

Pigeon pea due to its high protein content, it is desirable to use as a protein supplement to cereal-based diets. Its seeds show lower lipid content and are free of cholesterol and possess different minerals and vitamins. (Sebastia *et al*., 2001; Deka and Sarkar, 1990; Khandelwal *et al*., 2009; Elhardallou and Walker, 1994).

The test materials, the raw materials for the formulation of complementary food were yellow maize and green pigeon pea seeds and were purchased from Umuahia Central Market, Ubani Ibeku. Laboratory and other facilities were obtained from Ceslab Global Services Analytical Laboratory, Umuahia. Traditional fermentation method was applied was used.

The composite complementary food flours were formulated by gravimetric combination of the seed flour. The blends were subjected to the following Microbiological Analysis to include; Determination of Microbial Load, Determination of Microbial Flora, Characterization of Fungi Isolates, Characterization of Bacterial Isolates, Identification of Isolates and Determination of Coliforms

The result of the microbial load showed that there were significant variations in the microbial load of the different flours with fermented maize recording highest value ($1.52x10^5$cfu/g).

The occurrence of bacteria species in the raw material (flours) used showed 100% occurrence of yeast and *Aspergillus* in all the four samples, 75% occurrence of *Penicillium* in three samples (Raw maize, Raw PP and fermented maize) and 25% of Rhizopus in Raw PP.

The occurrence of fungi isolate showed differences in the levels of occurrence of the four species of fungi (mold and yeast) found in the flours.

The result of microbial load of the complementary foods based on fermented maize and pigeon pea flours showed a significant differences ($P<0.05$) in the microbial loads (fungi and bacteria) of the raw materials component used in the fermentation of the food blends (fermented maize and roasted pigeon pea).

The occurrence of bacterial isolates in the complementary foods indicated carryover of *microorganisms* in the raw materials (fermented maize and roasted pigeon pea) to the compounded foods. *Piteous movables* was the best prevalent (40% occurrence) being present only in the fermented maize flour and the 50:50 blend of roasted pigeon pea and fermented maize.

The fungi flora of the complementary foods based on fermented maize and roasted pigeon pea flours showed that yeasts and *Aspergillus* were present in all food samples (100% occurrence) while *Peniciluim* was found in the 60:40 and 50:50 (maize-Pigeon pea) samples only.

5. Conclusion

In conclusion, The bacterial load of the fermented maize was higher ($1.52x10^5$cfu/g) than that of the raw seed flour ($6.90x10^4$cfu/g) whereas the bacterial load of the roasted pigeon pea seed flour was much lower ($4.77x10^4$cfu/g) than the raw pigeon pea seed flour ($9.19x10^4$cfu/g). A similar trend was observed in other microbial load (fungi, mold and yeasts) of the flour.

Recommendation

The study therefore recommended that microbial loads could be reduced through heat treatment as complementary food is usually boiled or treated with boiled water before consumption

6. References

1. Aletor, O. And Ojelabi, A. (2007). Comparative evaluation of the nutritive and functional attributes of some traditional Nigerian snacks and oil seed cakes. *Pakistan Journal of Nutrition* 6: 99-103.
2. Aletor, O., & Ojelabi, O. (2007). Nutritional quality and utilization of pigeonpea (Cajanus cajan) seed: A review. *Food Science and Nutrition*, 5(5), 395-408.
3. Dewey, K. G., & Brown, K. H. (2003). Update on technical issues concerning complementary feeding of young children in developing countries and implications for intervention programs. *Food and Nutrition Bulletin*, 24(1), 5-28.
4. Di Giovanni, C., De Pee, S., & Engle, P. L. (2005). Complementary feeding practices and the risk of protein-energy malnutrition in young children in developing countries. *Food and Nutrition Bulletin*, 26(2), 126-138.
5. Eltayeb, A. M., Ali, M. A., & El Tayeb, M. A. (2010). Effect of processing on the nutritional quality of pigeonpea (Cajanus cajan L.). *Food Science and Nutrition*, 8(6), 766-774.
6. *chnology*, 42(2), 275-282.
7. Kimmons JE, Dewey KG, Haque E, Chakraborty J, Osendarp SJ, Brown KH. Low nutrient intakes among infants in rural Bangladesh are attributable to low intake and micronutrient density of complementary foods. *J Nutr.* 2005;135:444–51.
8. Kopsell, D. A., Smith, J. C., & Mazza, G. (2009). Increase in nutritionally important sweet corn kernel carotenoids following mesotrione and atrazine applications. *Journal of Agricultural and Food Chemistry*, 57(19), 8679-8686.
9. Kumar, D., & Jhariya, N. A. (2013). Nutritional, medicinal and economical importance of corn: A mini review. *Research Journal of Pharmaceutical Sciences*, 2, 7–8.
10. Kumar, D., & Jhariya, N. A. (2013). Nutritional, medicinal and economical importance of corn: A mini review. *Research Journal of Pharmaceutical Sciences*, 2(1), 7-8.
11. Kumar, P., Pandey, A., & Singh, R. P. (2010). Fermentation: a novel food processing technique to improve the nutritional quality of foods. *Journal of Food Science and Technology*, 47(10), 2421-2437.
12. Kunde, J. (2000). The potential for pigeonpea export from Africa. *Agricultural Economics*, 22(2), 121-132.
13. Lai, C., & Guo, X. (2011). The potential of nanoparticles for biomedical applications. *Nanomaterials and Plant Potential*, 2(2), 181-194.
14. Leszek, J., & Vincent, J. (2012). The nutritional value of pigeonpea (Cajanus cajan): *A review. Food Science and Nutrition*, 10(6), 1681-1692.
15. Li, Y., Zhang, L., & Zhang, Y. (2007). Effects of lactic acid fermentation on the quality and safety of meat products. *Meat Science*, 76(3), 483-491.
16. Lo Monaco, M. (2003). Pigeonpea: A review of its production, improvement, and uses. *Food Reviews International*, 19(3), 265-302.
17. Lopez, R., Sadras, V. O., and Muchow, R. C. (1987). Photosynthetic characteristics of cowpea and pigeonpea under water stress. *Australian Journal of Plant Physiology*, 14(1), 133-143.
18. López-Martínez, M., Paredes-López, O., & Mateos-Aparicio, I. (2009). Carotenoids and fatty acid composition of maize kernels (Zea mays L.) as affected by genotype, environment and processing. *Food Chemistry*, 115(2), 523-529.
19. Luther, C.K., and Deway, K.G. (2003). Supplement nutrient composition for fortified complementary foods. Proposed nutrient composition to fortified complementary foods. *J. Nutri*. 133:30115- 30205
20. Lutter, C. K., & Dewey, K. G. (2003). Micronutrient fortification of complementary foods for young children in developing countries. *Food and Nutrition Bulletin*, 24(1), 29-43.
21. Lutter, C.K. and K.G. Dewey, 2003. Supplement nutrient composition for fortified complementary foods. Proposed nutrient composition for fortified complementary foods. *J. Nutr*., 133: 3011S-3020S.
22. Maltas, S., Pérez-Rubio, M. T., & de la Peña, M. L. (2011). Nutritional composition and functional properties of pigeon pea (Cajanus cajan) flour. *Food Research International*, 44(12), 3008-3015.
23. Manay N, Shakaksharaswany M (2006). Food Facts and Principles. 2nd edition. New Age International Ltd., India. Pp. 247-248.
24. Mangelsdorf, P. C. (1974). Corn: Its origin, evolution, and improvement. Belknap Press of Harvard University Press.
25. Mannino, D. M., Holguin, F., Greves, H. M., Savage-Brown, A., Stock, A. L., & Jones, R. L. (2004). Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey. *Thorax*, 59(3), 194-198.
26. Martorell, R., Schroeder, D.G., Rivera, J.A., Kaplowitz, H.J. (1995): Patterns of linear growth in rural guatemalar adolescent and children. *J. of Nutr*. 125 (Supple) 1060s-1067s. https://doi.org/10.1093/jn/125.suppl- 4.1060S.

27. Mejia, G. (2008). Nutritional composition of maize (Zea mays L.). *Journal of Food Composition and Analysis*, 21(7), 735-744.

28. Milind, P., & Isha, D. (2013). Genetic diversity and breeding of maize. Springer Science & Business Media.

29. Monte, C.M. and Giugliani, E.R. (2004). Recommendations for the complementary feeding of the breast child. *J. Pediatri*. 80(5):131-141

30. Morton, J. (1976). On recursive reference. *Philosophia Mathematica*, 4(1), 46-62.

31. Mosha TCE, Laswai HS, Tetens I. Nutritional composition and micronutrient status of home made and commercial weaning foods consumed in Tanzania. *Plant Foods Hum Nutr (Dordrecht, Netherlands)* 2000;55:185–205.

32. Motarjemi Y. Research priorities on safety of complementary feeding. *Pediatrics.* 2000;106:1304–5.

33. Motuma, A.A., Azeb, L., and Bekesh, G. (2016). Complementary feeding. Review of Recommendations, feeding practices and adequacy of homemade complementary food preparation in Developing countries – lesson from Ethiopia. *J. Food. Nutri*. 3:4

34. National Food and Nutrition Council of Nigeria (NFCNS). (2004). Nigeria national micronutrient status survey 2004. Abuja, Nigeria: NFCNS.

35. NFCNS., 2004. Nigeria Food Consumption and Nutrition Survey 2001-2003. *summary. iita*, pp: 24-46.

36. Nizar, M., Akhtar, M. S., & Ahmad, I. (2015). Effect of roasting on the physico-chemical, functional and nutritional properties of lentil flour. *Journal of Food Science and Technology*, 52(2), 676-684.

37. Nout MJR, Ngoddy PO (1997). Technological aspects of preparing affordable fermented complementary foods. *Food Control* 8:279-286.

38. Nout, M. J. R. (1993). Functional properties of proteins in legume seeds. *Food Reviews International*, 9(1), 1-58.

39. Nout, M. J. R., & Ngoddy, P. C. (1997). Breast-feeding and the risk of infection in the first year of life. European Journal of Clinical Nutrition, 51(Suppl 1), S23-S30.

40. Nwokolo, E. C. (1987). Proximate composition, mineral and amino acid contents of some tropical legumes. *Plant Foods for Human Nutrition*, 37(1), 49-63.

41. Nzewi, C. E., & Egbuonu, S. C. (2011). Effect of roasting on the antinutritional factors and nutritional quality of cowpea (Vigna unguiculata L. Walp.) flour. *Food Science and Nutrition*, 9(1), 109-116.

42. Ogunbanjo, A. O., Afolabi, O. O., & Ogunbanjo, A. O. (2017). Microbiological quality and aflatoxin contamination of complementary foods consumed by under-fives in Ibadan, Nigeria. *Food Control*, 79, 118-123.

43. Okoronkwo, C.U, Agoha, EE.C, Ogodo A.C, and Nwachukwu N.O (2014). Nutritional and physical properties of weaning food flour based on maize and African yam bean seeds. *Inter.J.Adv.Eng.Mgt* (*IJAEM*). (1)(6):34-37.

44. Okoronkwo, C.U., Udensi, E.A., Okereke, H.C and Nwachukwu, N.O. (2017). Physicochemical characteristics and antinutritional factors of fermented complementary foods based on maize pigeon pea flour. *J. Advan. Food Sci Tech*. 4(11):38-43

45. Okpala, C. C., & Okoli, C. (2011). Nutritional evaluation of pigeonpea (*Cajanus cajan L.*) flour as a functional food ingredient. *Food Science and Nutrition*, 9(1), 131-137.

46. Omemeu, A. M. (2011). Fermentation dynamics during production of ogi, a Nigerian fermented cereal porridge. *Report and Opinion*, 3, 8 – 17.

47. Omemu, J. O. (2011). Traditional fermented foods and beverages of Nigeria: Their nutritional and health benefits. *African Journal of Food Science*, 5(1), 1-6.

48. Omemu, V. O. (2011). Effect of fermentation on the microbial quality and nutrient composition of some commonly consumed weaning foods in Southwestern Nigeria. African Journal of Food Science, 5(12), 642-647.

49. Onabanjo, A. A., Afolayan, A. A., & Akingbala, J. A. (2008). Fermented foods and beverages in Nigeria: A review. *African Journal of Biotechnology*, 7(23), 3406-3415.

50. Onabanjo, O. O., Oguntona, C. R. B., MaziyaDixon, B., Olayiwola, I. O., Oguntona, E. B., and Dixon, A. G. O. (2008). Nutritional evaluation of four optimized cassava-based complementary foods. *Africa Journal of Food Science*, 2, 136 – 142.

51. Onis, M. de, & Blossner, M. (1997). Anthropometric indicators of nutritional status. In J. G. Waterlow, R. J. Martorell, & A. S. Semba (Eds.), The epidemiology of undernutrition (pp. 83-136). Oxford University Press.

52. Onuorah, C. U., & Akinjide, I. O. (2004). Effect of roasting on the physicochemical and functional properties of yam flour. *Journal of Food Science*, 69(1), 146-150.

53. Onyekwere, E. U. (2007): Development of MaizeBambara Groundnut Complementary Foods Fortified With Foods Rich in Calcium, Iron, Zinc and Pro-vitamin A. M.Sc. Dissertation Submitted to Department of Food Science and Technology, University of Nigeria, Nsukka.

54. Ortiz, R., Gómez, M. C., & García-Villalba, M. J. (2017). Effect of fermentation on carotenoids and antioxidant capacity of tomato juice. *Food Science and Nutrition*, 5(1), 106-113.

55. Oshodi, A. A., Adeola, O. A., & Ogunwole, O. O. (1985). Effect of germination on the nutrient composition of pigeonpea (Cajanus cajan L.). *Plant Foods for Human Nutrition*, 35(1), 1-19.

56. Oshodi, A.A., Olaofe, O. and Hall, G.M. (1985). Amino acid, fatty acid and mineral composition of pigeon pea (Cajanus cajan). *International Journal of Food Science and Nutrition* 3: 187-191.

57. Pal, S. K., Mandal, S. K., and Ghosh, S. K. (2011). Pigeonpea: A review of its traditional uses, phytochemistry, pharmacology, and toxicology. *Journal of Ethnopharmacology*, 135(1), 1-20.

58. Pan American Health Organization. Guiding principles for complementary feeding of the *breastfed child.* Geneva: World Health Organization; 2001.

59. Pratibha, G., Srinivas, I., Rao, K. V., et al. (2015). Impact of conservation agriculture practices on energy use efficiency and global warming potential in dryland farming systems of India. *Agronomy Journal*, 107(4), 1442-1452.

60. Purseglove, S.W. (1992). Tropical Crops: Monocotyledons. Vol. 2, Longman Group Ltd., London, 20-140.

61. Rafiya, S., Kabir, S., Ahmad, A., & Ahmad, I. (2016). Assessment of microbiological quality and aflatoxin contamination of complementary foods consumed by under-five children in rural areas of Pakistan. *Food Control*, 65, 163-168.

62. Rasane, P., Jha, A., and Kumar, A. (2015). Reduction in phytic acid content and enhancement of antioxidant properties of nutricereals by processing for developing a fermented baby food. *Journal of Food Science and Technology*, 52, 3219 – 3224.

63. Rasane, S., Gaikwad, S., Sontakke, S., & Jagtap, S. (2015). Aflatoxin contamination of weaning foods in rural areas of Sangli district, Maharashtra, India. *Food Control*, 52, 416-420.

64. Roney, J. (2009). The spread of maize in the Americas. *Journal of Archaeological Research*, 17(1), 3-46.

65. Saxena, K. B., Kumar, R. V., & Sultana, R. (2010). Quality nutrition through pigeonpea—a review. *Health*, 2(4), 1335-1344.

66. Saxena, K. B., Kumar, R. V., & Sultana, R. (2010). Quality nutrition through pigeonpea—a review. *Health*, 2(4), 1335-1344.

67. Sheth, Jagdish N., and Yogesh K. Dwivedi. "Consumer behavior in India: a postmodern perspective." Sage Publications India, 2006.

68. Sheth, M. and R. Dwivedi, 2006. Complementary foods associated with diarrhea. Indian J. Pediatr., 73: 61-64.

69. Sheth, M., Dwivedi, R. Complementary foods associated diarrhea. *Indian J Pediatr* **73**, 61–64 (2006).

70. Sloan, J.; Heiholt, J.; Iyer, H.; Metz, S.; Phatak, S.; Rao, S.; Ware, D. (2009). Pigeon pea: a multipurpose, drought resistant forage, grain and vegetable crop for sustainable southern farms. *Sustainable Agriculture Research & Education*, 8(1), 1-12.

71. Soris, V., & Mohan, V. (2011). Pigeonpea: A functional food for human nutrition. *Journal of Food Science*, 76(9), R149-R157.

72. Souframanien, S., Varshney, R. K., and Rao, C. V. S. (2003). Microsatellite development and application in pigeonpea (Cajanus cajan). *Genetics and Molecular Biology*, 26(3), 517-525.

73. Spielvogel, J. J. (2005). Western Civilization: A Brief History (5th ed.). Boston, MA: Wadsworth.

74. Subbarao, A., Rupa, T. R., and Raju, P. S. (2000). Drought tolerance in pigeonpea. Crop Science, 40(3), 788-795.

75. Sun, D., Li, Y., and Zhang, Y. (1995). Anti-inflammatory and wound-healing activities of pigeonpea (Cajanus cajan) leaves. *Journal of Ethnopharmacology*, 51(2), 147-154.

76. Temesgen, A. (2013). Microbiological quality of complementary foods prepared by mothers in Debre Berhan, Ethiopia. *Ethiopian Journal of Health Sciences*, 23(1), 43-48.

77. UNICEF., 2001. Childrens and Womens Rights in Nigeria: A Wake-up call. In: Situation Assessment and Analysis, 2001. A. Hodges, (Ed.). National Planning Commission, Abuja and UNICEF Nigeria Abuja.

78. Valenzuela, H. and Smith, J. (2002). Sustainable Agriculture Green Manure Crops. SA-GM-7. Cooperative Extension Service, College of Tropical Agriculture and Human Resources, University of Hawaii at Manoa.

79. WHO (2004): Global Strategy for Infant and Young Child Feeding World Health Organization, Geneva pp 1-9. Yadav, S.K. and Sehgal

80. WHO/OMS, (2000): Child and Adolescent Health and Development: Nutrition and Infant Feeding. Pan American Health Organization/WHO.Guiding Principles for Complementary Feeding of the Breastfed Child.

81. Woldt, Monica and Moy, Gerald G. 2015. Literature Review on Effective Food Hygiene Interventions for Households in Developing Countries. Washington, DC: FHI 360/FANTA.

82. World Health Organization (WHO). (2002). Guiding principles for complementary feeding of infants and young children. Geneva, Switzerland: WHO.

83. World Health Organization. (1998). Complementary feeding of young children in developing countries: A review of current knowledge. Geneva: World Health Organization.

84. World Health Organization. *Complementary feeding of young children in developing countries: a review of current scientific knowledge.* Geneva: World Health Organization; 1998.

85. Wu, J., Chen, X., & Zhang, L. (2009). The nutritional and medicinal properties of mung bean: A review. Food Science and Nutrition, 2(2), 181-194.

86. Wu, J., Wang, H., and Chen, X. (2009). Pigeonpea: A potential functional food for human health. *Food Science and Human Wellness*, 1(1), 1-10.

87. Zhang, L., Zhang, Y., & Liu, R. H. (2015). Bioactive components and health benefits of germinated cereals. *Comprehensive Reviews in Food Science and Food Safety*, 14(1), 107-124.