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PERCENTAGE DISTRIBUTION OF BACTERIAL ISOLATES IN RAW PAP SAMPLES ASSOCIATED WITH DIFFERENT CONDIMENTS USED IN FORTIFICATION AND BACTERIAL ANALYSIS OF COOKED AND COOKED-FORTIFIED PAP SAMPLES

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ABSTRACT

This study was to evaluate the bacterial isolates in pap consumed in Ikwo and Abakaliki L.G.A of Ebonyi State, Nigeria and also to assess the effects of post cooking fortification, source of raw pap, cooking and storage methods on the bacteriological status of pap (akamu) in Ikwo (East) and Abakaliki (North) Local Government Area of Ebonyi State, Nigeria. The culture media used in this study are nutrient agar, MacConkey agar, Eosin-methylene Blue agar, *Salmonella Shigella* agar, peptone water, Kovac's reagent. A total of 100 households having children under 24 months and whose babies are fed with pap as one of the weaning foods were visited and interviewed using a simple structured questionnaire within Ikwo and Abakaliki Local Government Areas, Ebonyi State. Exactly one hundred and twenty-two (122) samples of raw pap processed by the mothers and the vendors were collected and analyzed. Also, 50 samples of cooked-pap and 50 samples of cooked-fortified pap samples were collected from the mothers. Bacterial isolates were identified by morphological features (such as Gram-reaction, shapes, cell arrangement and motility) and biochemical reactions. The result of the percentage occurances of

the isolates in both maize-based and sorghum-based pap samples are 16.67 and 25.00% for *Klebsiella* spp, 11.11and 16.67% for *Escherichria* spp, 5.56 and 11. 11% is for *Bacillus* spp, 5.56 and 5.56% for *Enterobacter* spp and 0.00 and 2.78% for *Proteus* spp. The result also showed that with the exception of *Entrerobacter* spp which occurred in about the same level in both maize- based and sorghum-based pap samples, all other isolates occurred higher in the later than former. The results of the viable bacterial counts of cooked- pap samples equally showed that thirty-seven (37) out of fifty (50) samples analyzed had growth following overnight incubation of 10^3 dilutions inoculated by pour-plate method on nutrient agar, and the bacterial counts among the samples differed significantly ($p<0.001$) and least significant difference(LSD) is 1.29. The mean bacterial counts ranged from $1.50 \pm 0.71 \times 10^3$ cfu/ml to $15.50 \pm 0.71 \times 10^3$ cfu/ml. Comparison of the bacterial loads of cooked and cooked-fortified pap samples showed that the cooked- fortified samples had much more plate counts than their unfortified counterparts, even at different dilution levels (10^6cfu/ml and 10^3) respectively. The bacterial isolates associated with the condiments used are *Escherichia* spp isolated from sample fortified with crayfish and soybeans, *Salmonella* spp from samples fortified crayfish and powdered milk where as *Staphylococcus aureus* isolated from samples fortified with soybeans. Proper heat treatment can considerably reduce the bacterial content of pap, but an attempt to improve the nutritional quality through fortification, especially with local ingredients after cooking creates room for further contamination.

Keywords: Fortification, Raw pap sample, Cooked pap sample and Cooked-Fortified Pap Samples

INTRODUCTION

Pap (*Akamu*) is an acid fermented porridge or gruel made from maize (*Zea mays*), sorghum (*Sorghum bicolor)* or millet (*Pennisetum glaucum)* [1]. It is also known as *eko* (Yoruba), *agidi* (Igbo and Yala), *kamu* (Isha), *kafa* (Hausa), *kamu* (Kogi) in Nigeria; and *Koko* in Ghana. It could be served thick, watery or in semi-solid form as in *agidi*, *koko* (*akamu*) and *eko* kolobo respectively [2].

The uses of *akamu* are so much found in West African countries where it serves as the first native weaning food for infants, breakfast for school children and adults, a choice of food for the sick and also encourages or stimulates breast milk production in nursing mothers [3]. As a major weaning food, it is used to supplement or replace breast milk when a child reaches

about 4 to 6 months before being introduced to the family diet [4].

Fermentation of *akamu* most of the time is spontaneous but could as well is induced. Lactic acid bacteria mainly the four genera *Lactobacillus, Lactococcus, Leuconostoc* and *Pedicoccus* are the organisms mostly involved in cereal fermentation; though other bacteria and yeast have been implicated [5]. These organisms not only ferment, but enhance aroma and microbial stability of the final product.

One of the greatest challenges affecting millions of people, particularly children in developing countries is lack of adequate protein intake in terms of quality and quantity [1]. Cereals are generally of low nutritive value. This calls for the need to supplement them with locally available legumes that are high in protein to increase the protein of cereal legume blends [5]. Several traditional fermentations have been upgraded to high technology production system and this has undoubtedly improved the general well being of people as well as economy [6]. In addition to improving the nutritive value of akamu through cereal-legume formulations and reductions in nutrient loss during processing, local fortification also exist which is adopted by people to improve taste. For instance, the use of sugar, milk, chocolate, *kulikuli* (groundnut cake), *akara* or *kose* (fried beans or beans cake), fruits and seeds berry to dose off the sour taste [2].

Like other fermented food products *akamu* is believed to have prolonged shelf life, improved nutritional status, reduced risk of food borne illness and most especially beneficial health effects due to the inhibitory and modulating activities of the fermenting organism and their products [7]. However, in developing countries of which Nigeria is one, the beginning of the weaning process in humans has been associated with an increase in diarrhoea episodes as a result of consumption of contaminated weaning foods [8]. Reports from the same author show that children aged 4 to 24months are at the greatest risk of developing diarrhea from contaminated food and water. This is because between 4 and 6 months of age, weaning foods are usually introduced to babies thus, exposing them to food borne pathogens. A bothering issue is what could be the sources of contamination to such acid fermented product as *akamu*, which in addition to having low pH (below 4.0) receives heat treatment before consumption considering that both factors are detrimental to pathogenic microorganisms. (Omemu, 2010), [3], with reference to some indirect evidences

suggested that about 15-70% of all diarrhoea episodes may be associated with practices of food preparation, handling, storage and feeding methods. This part of the country has not given much attention to investigating the possible occurrence of pathogens in cooked *akamu* taking into consideration the method of preparation, storage practices and post-cooking fortification.

Objectives of the Research

The objectives of this research are to:

- Determine the bacterial isolates in pap consumed in Ikwo and Abakaliki L.G.A of Ebonyi State
- Assess the effects of post cooking fortification, source of raw pap, cooking and storage methods on the bacteriological status of pap (akamu).

MATERIALS AND METHODS

Areas of the Study

The study was carried out in Ikwo (East) and Abakaliki (North) Local Government Area of Ebonyi State (Figure 1). Ebonyi state is located in the South-Eastern part of Nigeria. It is bounded by Enugu State by West, Cross River State by the East, Abia State by the South and Benue State by the North, and is between longitude 7^0C 30^1N and latitude 60^0C 45^1E. Abakaliki, the state capital has a tropical climate with an average relative humidity of 75% and may reach 80% during rainy season. The vegetation characteristics are predominantly rainforest with atmospheric temperature of about 30^0C. The state enjoys two distinct seasons, rainy season (between April and October) and dry season (between November and March) respectively. The study areas experience water scarcity during dry season.

The areas have about 65% of their populace as farmers, and they cultivate large quantity of cereal especially rice and maize, the greater percentage of the consumed sorghum is gotten from the northern part of the country.

Pap (*akamu*) Samples

One twenty-two (122) raw pap samples from mothers and vendors, fifty (50) cooked and and fifty (50) cooked-fortified samples were collected from mothers only.

Culture Media

The culture media used in this study are nutrient agar, MacConkey agar, Eosin-methylene Blue agar, *Salmonella-Shigella* agar, peptone water, Kovac's reagent

Data Collection

A total of 100 households having children under 24 months and whose babies are fed with pap as one of the weaning foods were visited and interviewed using a simple structured questionnaire within Ikwo and Abakaliki Local Government Areas, Ebonyi

State. The questionnaire was used to collect information about the socioeconomic characteristics of the mothers, age of babies at introduction of weaning food, food preparation and handling practices, food storage practices, preferred weaning food and occurrence of diarrhea in the 1- 24 month old children [9].

Some of the household were paid impromptu visit during which their usual way of pap preparation, serving and storage of cooked pap was observed; and a flow diagram was made on the common methods of cooking pap using information obtained on-site [9].

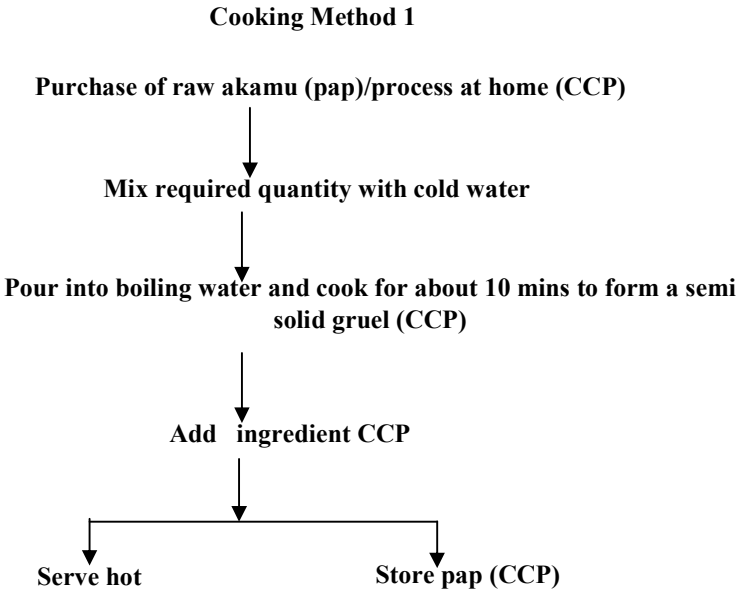

Figure 1: Observed pap (*akamu*) cooking methods in Ikwo and Abakaliki Local Government Areas (Method 1)

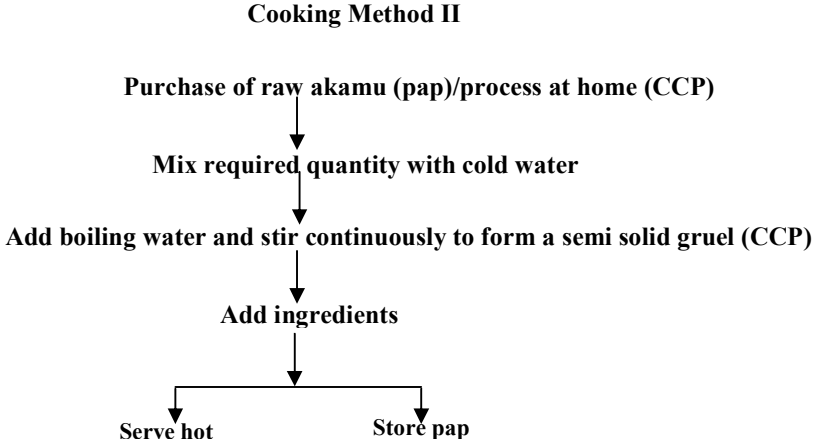

Figure 2: Observed pap (*akamu*) cooking methods in Ikwo and Abakaliki Local Government Areas (Method 2)

Collection of Samples

Exactly one hundred and twenty-two (122) samples of raw pap processed by the mothers and the vendors were collected and analyzed. Also, 50 samples of cooked-pap and 50 samples of cooked-fortified pap samples were collected from the mothers. The samples were put in sterile plastic containers with tight fitting lids labeled accordingly, and then taken to the laboratory for analysis within 5hrs of collection.

Enumeration of Microorganisms in the Samples

One (1) gram of each raw sample was aseptically weighed using weighing balance and dissolved in 10 ml of peptone water in a sterile test tube. One (1) loopful of each cooked sample (both fortified and unfortified) was also dissolved in 10 ml of peptone water and were incubated overnight. Ten-fold serial dilution was carried out on all the samples except fifty (50) raw samples used for analysis of percentage distribution of isolates in raw pap samples. A loopfull of each of the fifty (50) raw pap samples was inoculated on nutrient agar, MacConky agar, Eosin Mthylene Blue agar, *Salmonella Shigella* agar; and 1 ml of selected dilutions (,10^3,10^4,10^6 for cooked raw and cooked-fortified samples respectively) was inoculated into nutrient agar using pour plate method [9]. Total bacterial count were done on nutrient agar after overnight incubation at 37 ^{0}C whereas the colonies were picked and inoculated on Eosin Methylene Blue agar,MacConkey and *Salmonella-Shigella* agar for morphological examination. Also twelve raw pap samples were cooked using two cooking methods (I, II) during which temperatures and plate counts at different preparatory stages and storage were checked.

Identification of Isolates

Bacterial isolates were identified by morphological features (such as Gram-reaction, shapes, cell arrangement and motility) and biochemical reactions.

Statistical Analysis

Percentage, bar chart and analysis of variance (ANOVA) were used to analyze data obtained.

RESULTS

Percentage Distribution of Bacterial Isolates in Raw Pap Samples.

Bacterial isolates were detected in thirty-six (36) out of fifty (50) raw pap samples analyzed by inoculating a loopful of 1 g of each sample dissolved in 10 ml of peptone water. The percentage occurances of the isolates in both maize-based and sorghum-based pap samples are 16.67 and 25.00% for *Klebsiella* spp, 11.11 and 16.67% for

Escherichria spp, 5.56 and 11. 11% is for *Bacillus* spp, 5.56 and 5.56% for *Enterobacter* spp and 0.00 and 2.78% for *Proteus* spp. The result also showed that with the exception of *Entrerobacter* spp which occurred in about the same level in both maize- based and sorghum-based pap samples, all other isolates occurred higher in the later than former (Figure 3).

Bacterial Analysis of Cooked and Cooked-Fortified Pap Samples

These tests were conducted to determine the bacterial load of cooked and cooked-fortified pap samples. The results of the viable bacterial counts of cooked- pap samples showed that thirty-seven (37) out of fifty (50) samples analyzed had growth (Figure 4) following overnight incubation of 10^3 dilutions inoculated by pour-plate method on nutrient agar, and the bacterial counts among the samples differed significantly ($p< 0.001$) and least significant difference (LSD) is 1.29 . The mean bacterial counts ranged from $1.50 \pm 0.71 \times 10^3$ cfu/ml to $15.50 \pm 0.71 \times 10^3$ cfu/ml (figure 4).

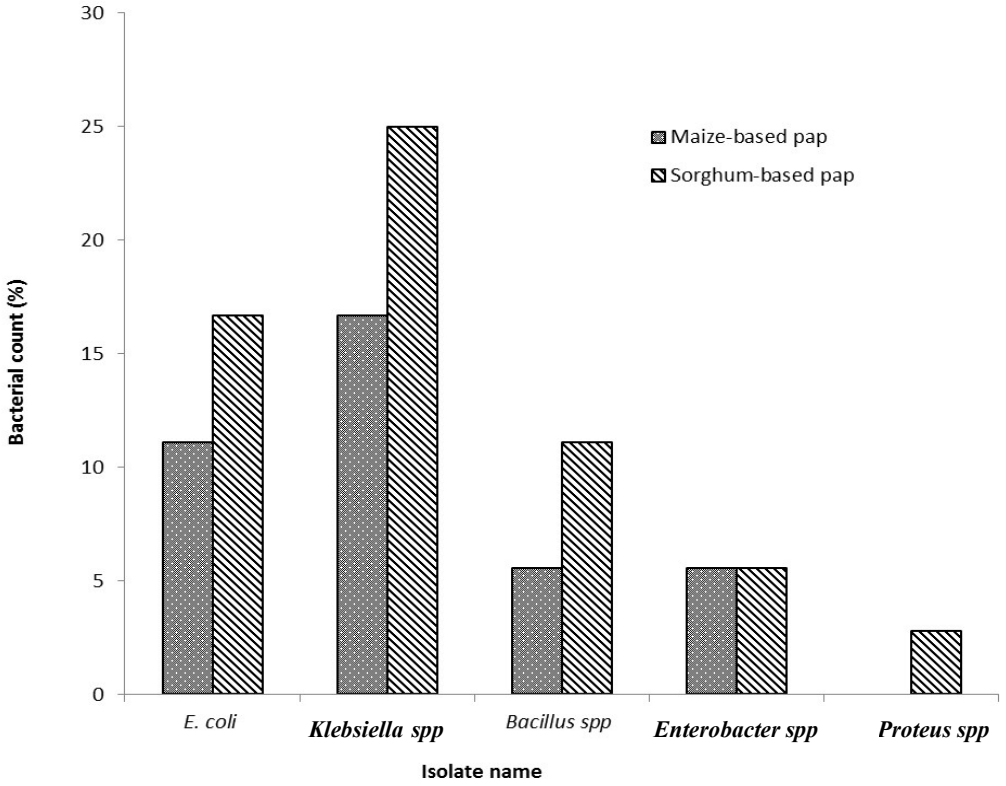

Figure 3: Percentage Distribution of Bacterial Isolates in Raw Pap Samples

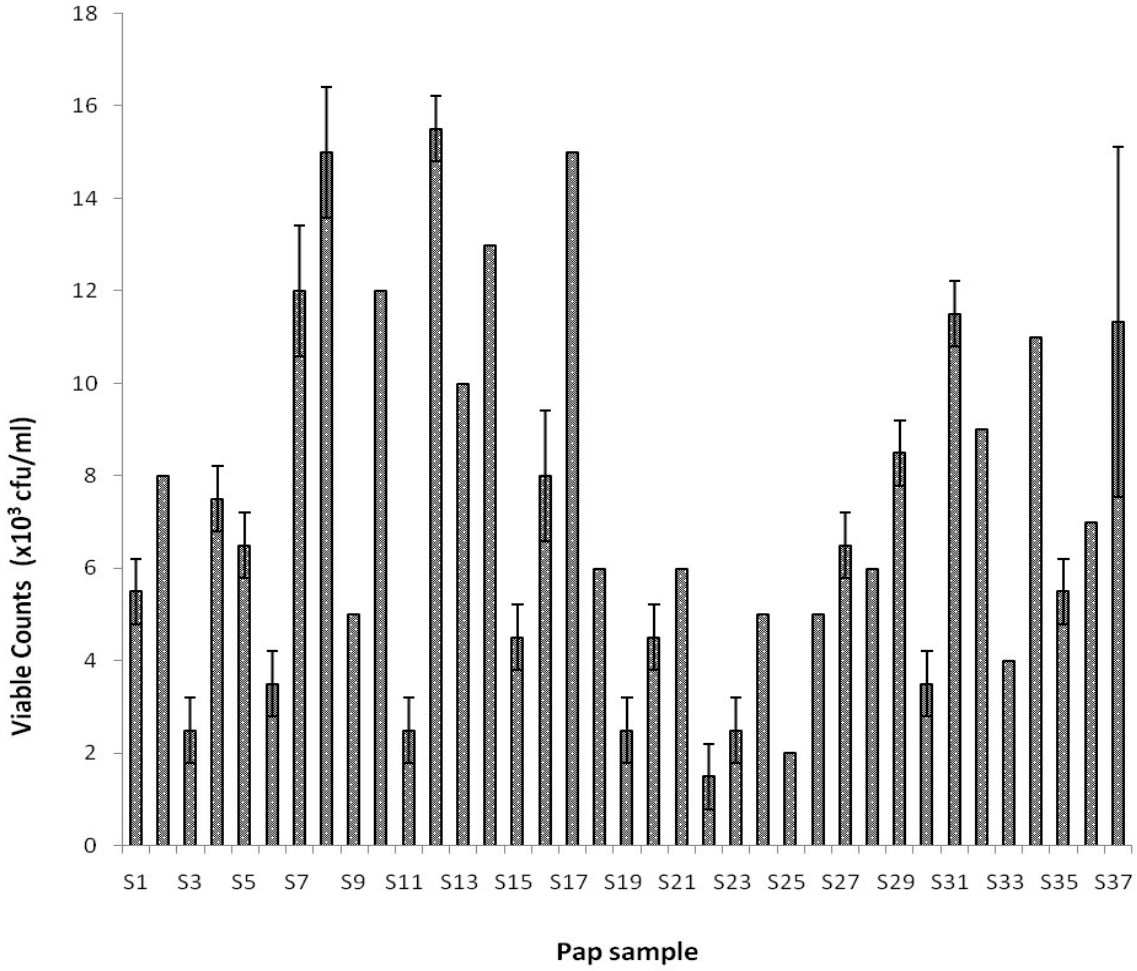

Figure 4. Viable bacterial counts in cooked pap samples

The results of the fifty (50) cooked-fortified samples also showed significant differences in viable counts ($p<0.00l$) and least significant difference (LSD) is 2.07. The mean viable count ranged from 2.50 ± 2.90 x 10^6 cfu/ml to 40.00 ± 0.00 x 10^6 cfu/ml. Increment in viable counts occurred in samples fortified with local ingredients, specifically crayfish (27.00±0.00, 32.00 ± 0.00, 37.30 ± 3.37, 38.00± 7.89) x 10^6 cfu/ml and soybeans (14.00 ± 1.07, 29.00 ± 0.00, 31.25 ± 5.42, 34.13±4.36,35.00 ± 0.00)x 10^6 cfu/ml, while no bacterial count was observed in samples fortified with commercial ingredients, oil and honey except for powdered milk which had 2.50± 2.70 x 10^6 cfc/ml in only one sample (figure 5).

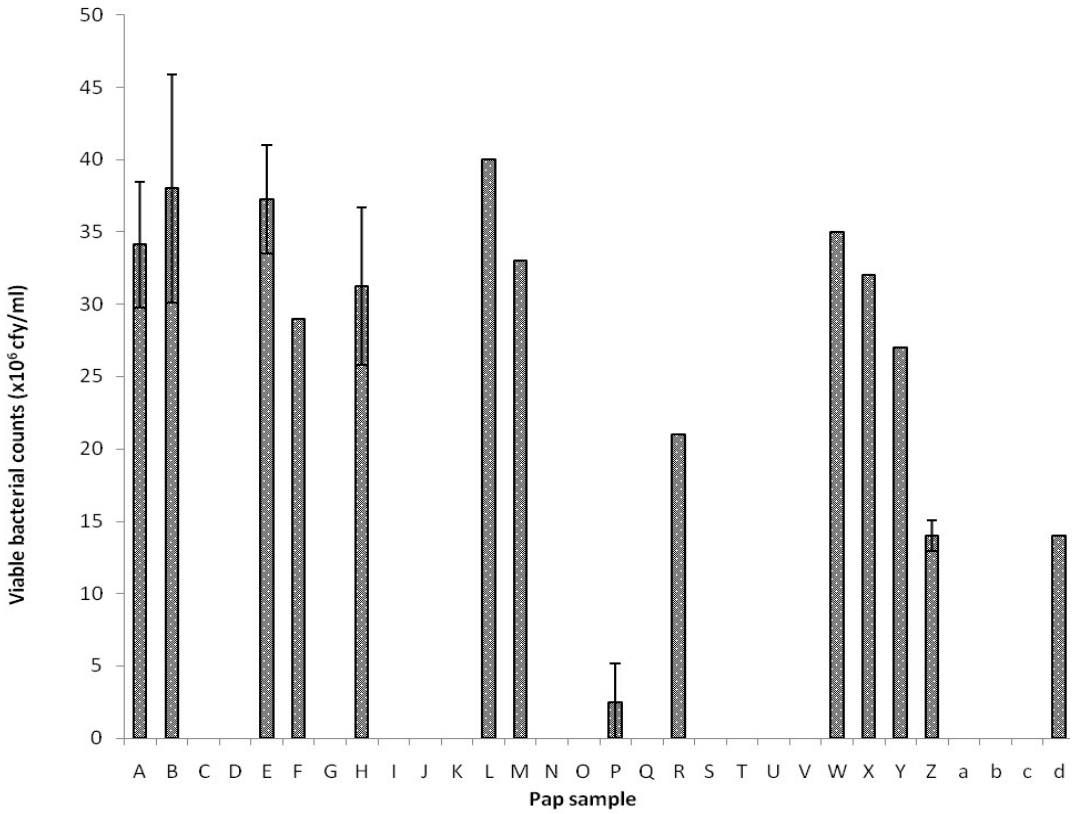

Figure 5. Mean bacterial counts in cooked-fortified pap samples

Comparison of the bacterial loads of cooked and cooked-fortified pap samples showed that the cooked- fortified samples had much more plate counts than their unfortified counterparts, even at different dilution levels (10^6cfu/ml and 10^3) respectively. The difference is as shown in figure 6.

Also, of interest in this work is the pattern of contamination shown by different fortifications. All the samples fortified with commercial ingredients but one (sample 16 whose mean bacterial counts is 2.5 x 10^6 cuf/ml) had no bacteria while most samples fortified with local ingredients showed very high bacteria load (Figure 6). Significant differences were also observed among various local ingredients used; sample with crayfish ranking highest in mean plate counts (40.0038.00, 37.25,32.00 and 27.00) x 10^6 cfu/ml followed by those fortified with soybeans (34.13.35.00, 33.00, 21.00, 14.00) x10^6cuf/ml. The discrepancies in the mean plate counts of samples fortified with different local ingredients are in the processing and packaging of these products. For instance, most commercial ingredients are canned unlike the local ones like crayfish that is so much exposed to environmental contaminations and possibly pathogens being shed by human carriers, including the normal

flora which is on arrival to new location assume pathogenic activities or roles. This is in line with the assertion of Omemu and Aderoju (2008), [10], that mishandling and disregard of hygienic measures on the part of food vendors may enable pathogens to come in contact with foods and in some cases to survive and multiply in sufficient numbers to cause illness in the consumer.

The bacterial isolates associated with the condiments used are *Escherichia* spp isolated from sample fortified with crayfish and soybeans, *Salmonella* spp from samples fortified crayfish and powdered milk whereas *Staphylococcus aureus* isolated from samples fortified with soybeans.

In **Table 1**, *Escherichria* spp and *Staphylococcus aureus* were isolated from samples fortified with soybeans, *Escherichria* spp and *Salmonella* spp from samples fortified with crafish whereas powdered milk had only *Salmonella* spp.

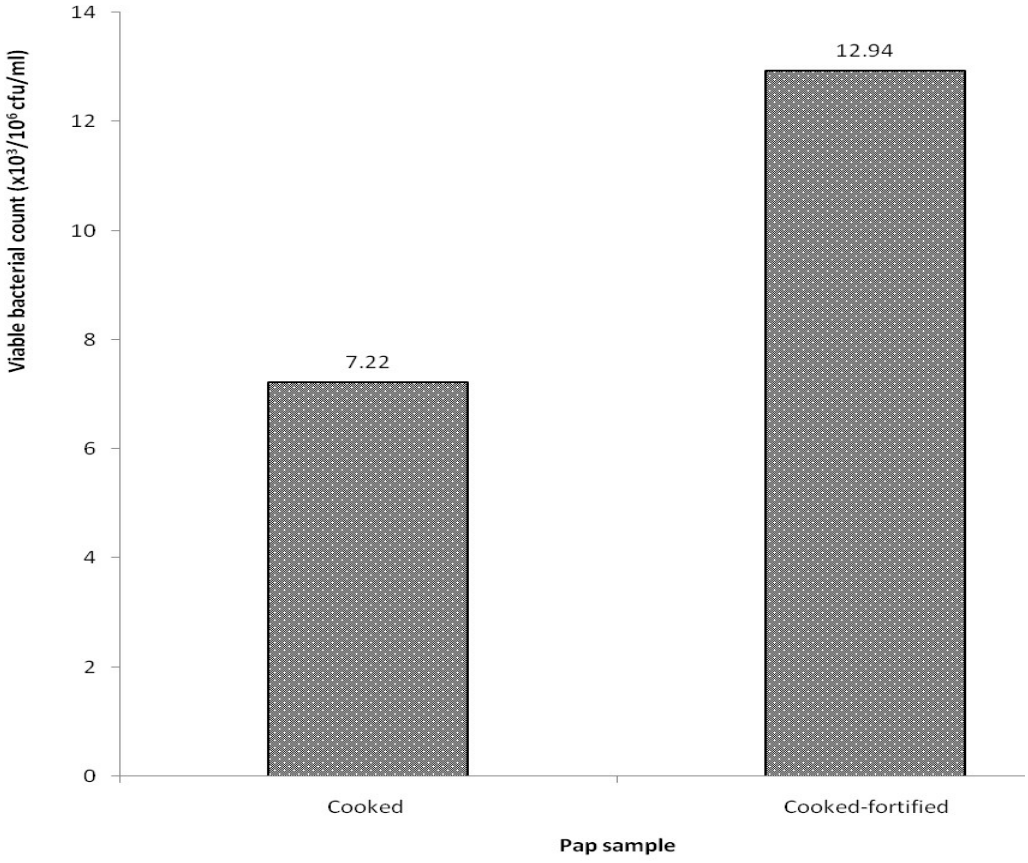

Figure 6. Comparison of mean bacterial counts in cooked and cooked-fortified pap samples. Values of bacterial counts for cooked pap samples are in 10^3 while those for cooked-fortified samples are in 10^6

Table 1: Bacterial isolates associated with different condiments used in fortification of Pap Samples

	BACTERIAL ISOLATE SOURCE					
	Ground Crayfish	**Ground Soybeans**	**Powered Milk**	**Tea**	**Sugar**	**Red Oil**
***Klebsiella* spp**	-	-	-	-	-	-
***Escherichria* spp**	+	+	-	-	-	-
Staphylococcus aureus	-	+	-	-	-	-
***Salmonella* spp**	+	-	+	-	-	-

Key: + = positive; - = negative

DISCUSSION

Pap (*akamu*), like most other food, is not all the time pathogen free irrespective of its high probiotic potential (lactic acid fermented product). It therefore becomes imperative that the microbiological status of such important food (major weaning food) be assessed from time to time considering the position it occupies for every average Nigerian and Africa at large.

The results obtained in percentage distribution of bacterial isolates in raw pap samples showed that *Klebsiella* spp (16.67 and 25.00) had the highest percentage occurrences in both maize-based and sorghum-based pap samples followed by *Escherichia* spp (11.11 and 16.67), *Bacillus* spp (5.56 and 11.11)5 and *Enterobacter* spp (5.56 and 5.56%) while *Proteus* spp (0.00 and 2.78%) had the least percentage occurrence. Olasupo, (2002), [11], share the same view; that among microorganisms of public health importance, akamu and kunu zaki contained *Bacillus subtilis*, *Klebsilla* spp, *Staphylococcus aureus* and *Enterococcus feacalis*. Kunene, (1999), [8], also reported the presence of *Escherichia coli* and *Bacillus cereus* in fermented sorghum meal. The level of occurrence of these isolates reflects the fact that though Gram positive bacteria like *Bacillus* spp are more resistant to low pH which favours spore formation, [12 and 13], had it that survival of organism in challenge test (that is, exposure of organisms to harsh environmental conditions eg low pH) depends on other factors such as the species and strains, initial inoculum, quantity and speed of acid production, storage temperature and time. Thus, the *Coliforms-Klebsiella* spp and *Escherichia* spp may have grown to high levels during early stage of fermentation and finally developed resistance through acid tolerance response (ATR) mechanisms according to [14].

This work further shows that there are significant differences ($p<0.001$) in the occurrences of the isolates in maize-based

and sorghum-based samples respectively except for *Enterobacter* spp (5.56 and 5.56%) shown in figure 3. This agrees with the report of Oyarekua (2011), [15], that plate counts differed significantly between millet/cowpea and millet/sorghum combination due to solubilization of protein since millet is richer in protein than sorghum and Bolaji, (2010), [1], reported that sorghum is richer in protein than maize.

Pap is generally believed to be of low nutritive quality coupled with nutrient loses associated with fermentation processes. Thus, there have been several attempts at improving the nutritional quality of pap. One of the common means of achieving this is fortification, which though remedies the situation, has been revealed by this work to be accompanied by isolates that contaminate the food. So, fortification is identified as a Critical Control Point (CCP). There are appreciable differences ($p<0.001$) in bacterial counts of cooked pap and cooked fortified pap samples even at different dilutions (10^3 and 10^6) respectively (Figure 6). The mean bacterial counts of cooked pap samples ranged from 1.50 x 10^3 cfu/ml to 15.50 x 10^3 cfu/ml with only few samples 17, 8, 14, 7, 31, 37 and 34 recording significant counts (Figure 5). This is unlike the mean bacterial counts of cooked-fortified samples which ranged from 2.50 x 10^6cfu/ml to 40.00x10^6cfu/ml with many samples 16, 26, 30, 19, 8, 24, 23, 5, 2 and 12 having very high counts (Figure 6). This agrees with the report given by Adesokan. (2011), [4], that there was significant increase in the bacterial load of fortified pap samples compared to unfortified ones; which could be due to enrichment of sample by ingredients.

Pap (*akamu*) is susceptible to bacterial contamination irrespective of its probiotic quality (lactic acid fermented product). The organisms *Klebsiella* spp*, Escherichia* spp*, Bacillus* spp*, Enterobacter* spp and *Proteus* spp were isolated from raw pap samples while *Staphylococcus aureus, Salmonella* spp and *Escherichia* spp were introduced by ingredients. Proper heat treatment can considerably reduce the bacterial content of pap, but an attempt to improve the nutritional quality through fortification, especially with local ingredients after cooking creates room for further contamination.

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