IJBPAS, December, 2016, 5(12): 3359-3374

ISSN: 2277–4998

International Journal of Biology, Pharmacy and Allied Sciences (IJBPAS)

'A Bridge Between Laboratory and Reader'

www.ijbpas.com

BACTERIAL QUALITY OF VENDED AND SELF-PREPARED PAP SAMPLES AT DIFFERENT PREPARATION STAGES AND STORAGE IN IKWO (EAST) AND ABAKALIKI (NORTH) LOCAL GOVERNMENT AREA OF EBONYI STATE, NIGERIA

[1]NWORIE OKORO, [2]ORJI JERRY O., [2]OGBUAGALABA GLORIA N., [3]ONU EUSLAR NNENNA, [1]UZOH CHUKWUMA VICTOR, [1]ONYEKWERE AMOS MATTHIAS AND [2]AGWU CHUKWUEMEKA LOVE

[1]Department of Biology, Microbiology and Biotechnology Faculty of Science, Federal University Ndufu-Alike Ikwo, Nigeria

[2]Department of Applied Microbiology, Ebonyi State University, Abakaliki, Nigeria

[3]Ebonyi State School of Health Mgbo, Ohaukwu Local Government Area, Ebonyi State, Nigeria

ABSTRACT

This study was designed to determine the bacterial isolates in pap consumed in Ikwo and Abakaliki L.G.A of Ebonyi State and 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. 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. Bacterial isolates were identified by morphological features (such as Gram-reaction, shapes, cell arrangement and motility) and biochemical reactions. The result of the vended samples had significantly ($p<0.00l$) higher mean bacterial counts (20.46 ± 5.75) x 10^4 cfu/ml than household prepared samples (7.38 ± 3.23) x 10^4 cfu/ml. The results of the preparatory practices observed by the mothers showed that most 86(89.58%) use cooking

method II. Percentage analysis of the data gotten using questionnaires also showed that children studied have the age range of 1 – 24 months (weaning age). Most of the mothers 90 (93.95%) are within active working age. Very few 4 (4.17) had no formal education. A good number 40 (41.67%) work with the government, 38 (39.58%) are business women while few 18 (18.75%) are house wives. The critical control points identified from handling of pap (*akamu*) are purchase of raw pap from unreliable sources, inadequate heat treatment, in addition of condiment after heat treatment and over storage of both raw and cooked pap samples while the associated hazards include bacterial isolates and their growth. 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: Pap, Cooking fortification, Bacteriological status and Vendors

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.
- To 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].

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.

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

Bacterial Analysis of Vended and Self-prepared Pap Samples

The difference in bacterial counts of vended pap (*akamu*) and those prepared in various households (self-prepared) are presented figure 1. The vended samples had significantly ($p<0.00l$) higher mean bacterial counts (20.46±5.75) x 10^4 cfu/ml than household prepared samples (7.38±3.23) x 10^4 cfu/ml (Figure 1); and most 64 (66.67%) of the pap used in study areas were sourced from vendors.

Bacterial Quality of Pap at Different Preparation Stages and Storage

The mean temperature of cooked and stored pap were 100 ^{0}C and 44 ^{0}C ; 83^{0}C and 36^{0}C respectively. Both cooking methods reasonably reduced the bacterial load immediately after cooking as shown in figure 2.

Storage Effect on the Bacterial Quality of Pap

Increase in bacterial counts was observed after storage length of 6 hours Table 1 which presents results on storage and hygienic practices observed by the

mothers during pap preparation showed that higher percentage (59.50%) of the mothers store raw pap locally(compressing in a can with addition of water on top) while 37.50 % refrigerate., 58.33% store raw pap above two (2) days., 46.88% store cooked pap in food warmer/flask up to 6hrs and more percentage (43.75%) of mothers do not re-heat the pap before feeding their babies.

In table 1, raw and cooked pap samples in the study areas are stored above 2 days and 6 hours respectively. Ingredients are also mostly added after heat treatment.

Profile of Mothers and Children Studied

Percentage analysis of the data gotten using questionnaires showed that children studied have the age range of 1 – 24 months (weaning age). Most of the mothers 90 (93.95%) are within active working age. Very few 4 (4.17) had no formal education. A good number 40 (41.67%) work with the government, 38 (39.58%) are business women while few 18 (18.75%) are house wives.

In table 2, the babies studied are between 1-24months, most (50%) of mothers are between 21-30 years old. Only 4.2% had no formal education while 38.5% had post secondary education. Up to 41.7% are civil servants, 39.6% are business women and 18.7% are full house wife.

Identification of Hazards and Critical Control Points (CCP) during Pap (*akamu*) preparation

The critical control points identified from handling of pap(*akamu*) are purchase of raw pap from unreliable sources, inadequate heat treatment, in addition of condiment after heat treatment and over storage of both raw and cooked pap samples while the associated hazards include bacterial isolates and their growth (table 3). In table 3, the summary of the hazards and critical control points (CCPs) identified during household preparation of pap and storage.

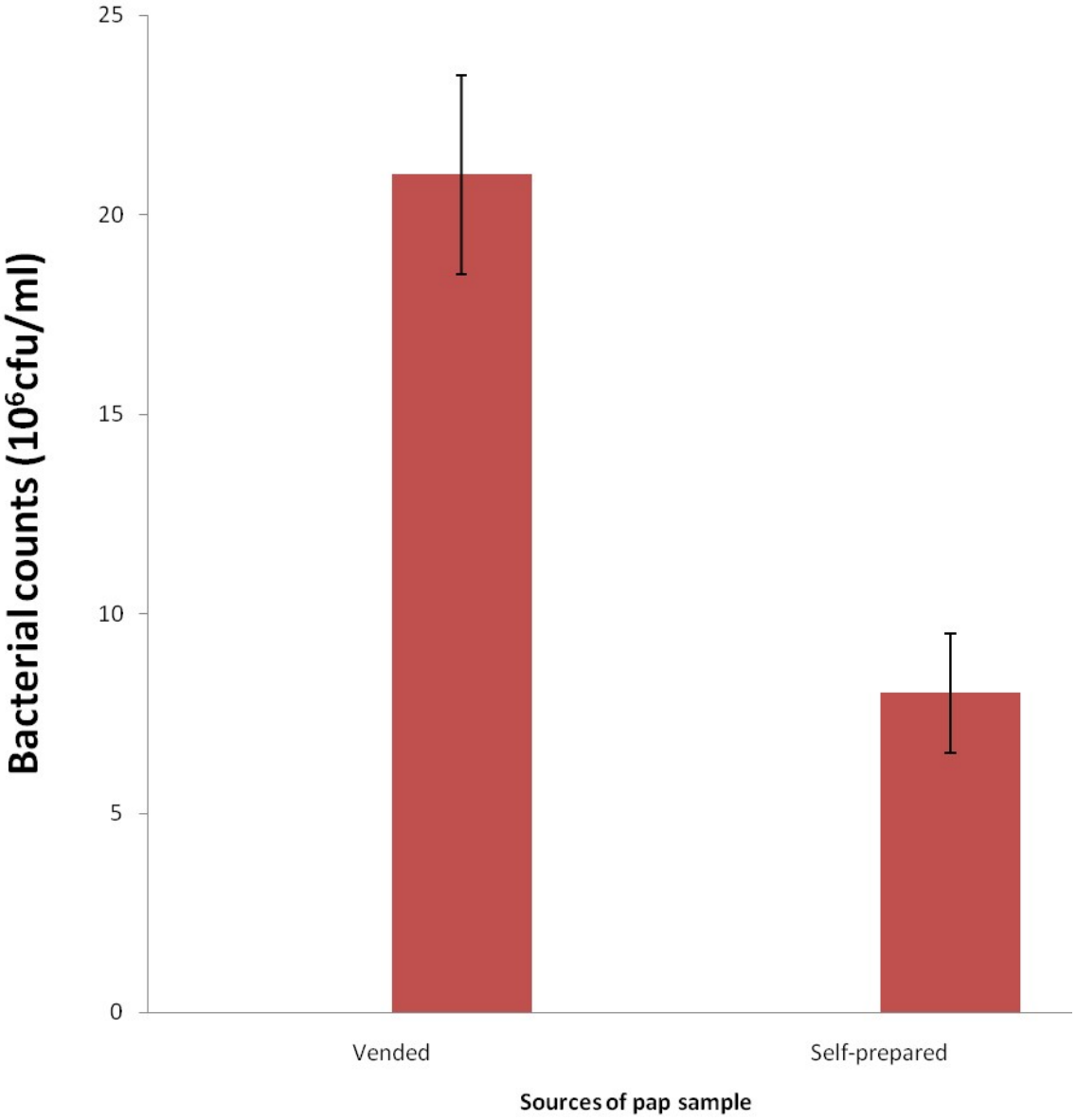

Figure 1: Comparison of mean bacterial counts of vended and self-prepared raw pap samples from Ikwo and Abakaliki L.G.As, Ebonyi State.

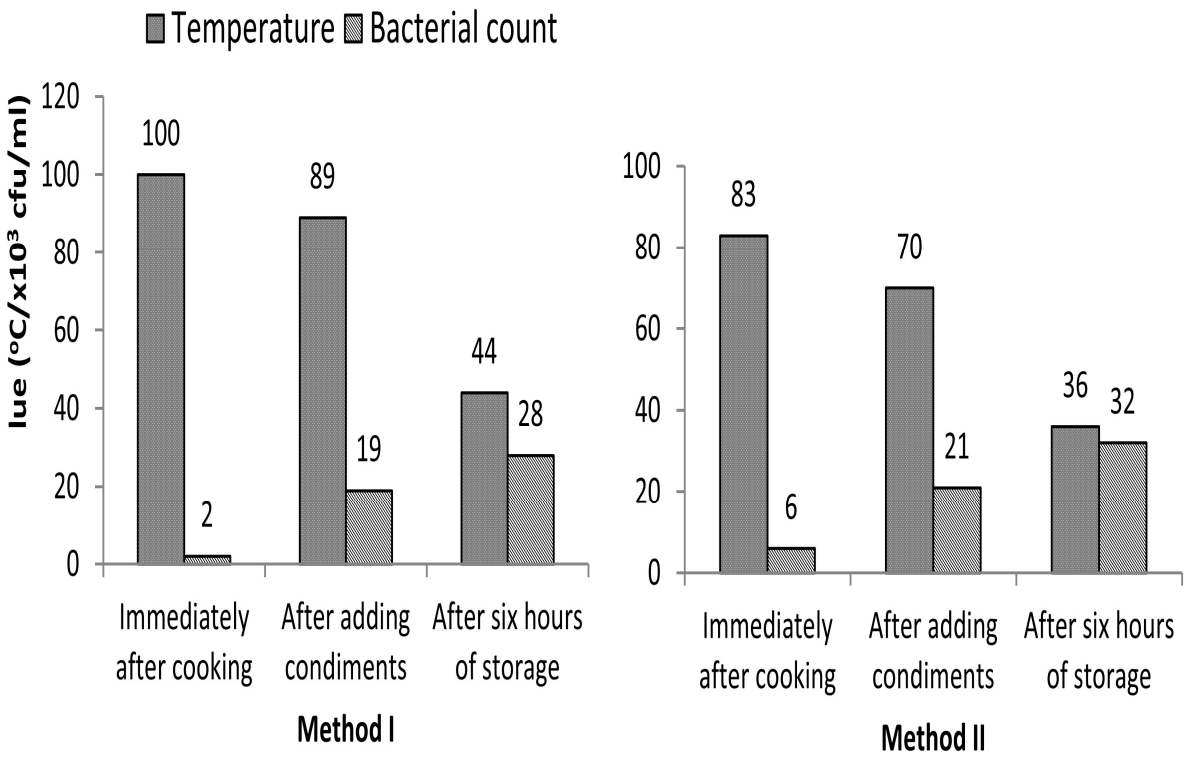

Figure 2: Temperature (^{o}C) and bacterial loads (x10^{3} cfu/ml) of pap prepared by two different methods after six hours of storage

Table 1: Storage and Hygienic Practices during preparation of Pap

PARAMETERS	NUMBER OBSERVED	PERCENTAGE (%)
Storage of Raw Pap		
Refrigeration	**36**	**37.50**
Compress in can and pour water on top	**57**	**59.50**
Storage Period of Raw Pap		
One day	**17**	**17.71**
Two days	**20**	**20.83**
Above days	**56**	**58.33**
Storage Method of Cooked Pap		
Refrigeration	**9**	**9.38**
In food flask/warmer	**63**	**65.63**
In bowel at room temperature	**14**	**14.58**
Storage Period of Cooked Pap		
1-2hours	**25**	**26.04**
3-6hours	**15**	**15.63**

1 day	**45**	**46.88**
Post-Cooking Heat Treatment		
Usually	**16**	**16.67**
Sometimes	**38**	**39.58**
No re-heating	**42**	**43.75**
Hand Washing Practices		
Before food, preparation	**65**	**67.71**
After toilet use	**31**	**32.29**
Utensil Cleaning Practices		
Cold water with soap always	**44**	**45.83**
Hot water with soap sometimes	**10**	**10.42**
Cold water only	**42**	**43.75**
Diarrheal experience		
During weaning	**78**	**81.25**
No diarrhea during weaning	**14**	**14.53**
Frequency of diarrhoea		
One episodes	**38**	**39.58**
> two episodes	**34**	**35.42**

Table 2: Profile of the Mothers and Children Studied (R =100)

PARAMETERS	NUMBER OBSERVED	PERCENTAGE (%)
Gender of children		
Male	**45**	**40.63**
Female	**51**	**53.13**
Age of Children		
1-6months	**62**	**64.58**
7-24 months	**34**	**35.42**
Age of Mothers		
21-30	**48**	**50.00**
31-40	**42**	**43.75**
Above 40	**6**	**6.25**
Educational Status of Mothers		
No formal education	**4**	**4.17**
Primary school	**12**	**12.50**
Secondary school	**3.3**	**34.38**
Post secondary	**37**	**38.45**
Occupation of Mothers		
Government workers	**40**	**41.67**
Business	**38**	**39.58**
House wives	**18**	**18.75**

Table 3: Identification of Hazards and Critical Control Points during Cooking and Storage of Pap (*akamu*)

STEPS	HAZARDS	CCP
Purchase of raw pap	**Bacterial isolate**	**Purchasing raw pap from unreliable sources**
Cooking	**Bacterial isolate**	**Inadequate heat treatment**
Addition of condiments	**Growth of bacterial isolate**	**Addition of contaminated condiments after heat treatment**
Storage	**Growth of bacterial isolate**	**Storage at room temperature up to 6hours**

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.

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.

Cooking of food improves the taste, smell, appearance, and digestibility and most importantly reduces microbial load. Thus, heat treatment is identified as Critical Control Point (CCP), since it is aimed at enhancing the keeping quality and improving the overall safety of food. Furthermore, two cooking methods were observed viz: one (method 1) which is more effective than the other (method II) (Table 1). Unfortunately, unlike the report of Omeumu and Omeike (2010), [9], where most Lagos mothers used cooking method I (pouring stirred raw

pap into boiling water and cooking for about 10 minutes further), 89.58% of the mothers in this study used cooking method II (pouring boiling water into stirred pap). This observation therefore, is in line with the assertion of Lei, (2006), [10], that though Nigerian mothers claim to prefer feeding their children with food served from their kitchen for the reason of being warm and pathogen-free, such foods may not attain temperature high enough to kill or reduce the pathogen to bearable minimum and ingredients are mostly introduced after heat treatment.

Evidences abound that lactic acid fermented products like *akamu* (pap) inhibit the growth and survival of pathogenic bacteria. However other studies have shown that improper storage of pap at home before and after cooking encourages growth of pathogens [11].

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. 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. 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.

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. 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) x10^6 cuf/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 Omeike, (2010), [9], 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 results of the bacterial quality of vended and household (self prepared) raw pap samples as investigated using analysis of variance (ANOVA) showed that vended samples outweighed the self-prepared in mean bacterial counts. Also, results of the survey carried out in this study showed that most of the mothers studied are educated and about one- third of them are civil servants. This has significant implication for child health in general and for food hygiene behavior particularly since education and employment both influence access to household facilities, including those related to food hygiene and environmental health [12]. However, most of the mothers purchase raw pap and ingredients from market vendors due to time lack, the practice that also poses considerable health risk and so also identified as a Critical Control Point (CCP) in this work. This is in agreement with the work of Omemu and Omeike, (2010), [9], that most Lagos and Abiokute mothers prefer using vended pap because the processing of pap is long and tedious, and many studies, including that of Omemu, (2005), [3], have shown that vended foods are positive vectors of food borne illness.

Results of this study equally showed that higher percentage of the mothers store raw pap locally, store raw pap above two days, store cooked pap in food warmer/flask for up to 6 hours and do not

reheat the pap before feeding the children (Table 2); and analysis of bacterial quality at different preparatory stages proved that prolonged storage affects level of contamination. This may be associated with practices of food preparation, handling, storage and feeding method.

The Critical Control Points (CCP) identified in this study includes purchase of raw pap from vendors, insufficient heat treatment, improper storage and post cooking fortification.

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.

REFERENCES

[1] Bolaji, O. A., Olubunmi, O. A. and Samuel, A. G. (2010). Quality assessment of collected cereal-soybean mixtures in "ogi" production. *York Science Journal New.* 3 (10): 17-26.

[2] Afolayan, M. O., Afolayan, M. and Abuah, J. N. (2010). An investigation into sorghum-based ogi storage characteristics. *Advanced Journal of Food Science and Technology* . 2 (1):72-78.

[3] Omemu, A. M., Edema, M. O. and Bankole, M. O. (2005). Bacteriological assessment of street vened ready to eat (RTE) vegetables and pre-packed salad in Nigeria. *Nigerian Journal of Microbiology*. 19 (1-2): 497-504.

[4] Adesokan, I. A., Fawole, A. D., Ekanola, Y. A., Odejayi, D. O. and Olanipekun, O. K. (2011). Nutritional and sensory properties

of soybean fortified composite ogi-a Nigerian fermented cereal gruel. *African Journal of Microbiology Research* 5 (20): 3144-3149.

[5] Salovaara, H. (2004). Lactic acid bacteria in cereal-based products, 3rd ed. New York: Marcel Dekker Inc. Pp 340-346.

[6] Achi, O. K. (2005). The potential of upgrading traditional fermented foods through biotechnology. *African Journal of Biotechnology* 4 (5):375-380.

[7] Caldron, M., Loiseau, G. and Guyot, J. (2003). Fermentation by Lactobaciillus fermentum of ogi of different combinations of carbohydrates occurring naturally in cereals: consequences on growth energetic and alpha-amylase production. *International Journal of Food Microbiology*. 80**:** 161-169.

[8] Kunene, N. F., Genrnaras, I., Von-Holy, A. and Hastings, J. W. (2000). Characterization and determination of origin of lactic acid bacteria from a sorghum-based fermented weaning food by analysis of soluble proteins and amplified garment length polymorphism finger printing. *Applied and Environmental Microbiology*. 66: 1084-1092.

[9] Omemu, A. M. and Omeike, S. O. (2010). Microbiological hazard and critical control point identification during household preparation of cooked ogi used as weaning food. *International Food Research Journal*. 17: 257-266.

[10] Lei, V. (2006). Probiotic potential of African fermented millet. *Journal of Applied Microbiology*. 96:384 – 397.

[11] Charalampopoulos, D., Wang, R., Pandiella, S. S. and Webb, C. (2002). Application of cereal and cereal component in functional foods. *International*

Journal of Food Microbiology. 79**:** 131-141.

[12] Mortajemi, Y. and Asante, A. (2002). Impact of small scale fermentation technology on food safety in developing countries. *Internal Journal of Food Microbiology*.75: 213-229.