

# Isolation and Identification of Microorganisms Associated with Pap and Stored Corn Starch.

## ABSTRACT

This study isolated and identify microorganisms associated with pap and stored corn starch. This was done to help portray spoilage microorganisms associated with stored corn starch and pap. The study was achieved through laboratory analysis of pap sample and stored sample over a period of 7days. Result obtained shows that the pap samples are contaminated with bacteria. The profile of bacteria evaluated shows an increasing trend over the retention period with isolates of bacteria such as *Pseudomonas sp.*, *Bacillus spand Lactobacillus sp.* which are not entirely eliminated when prepared in heat (pap). The isolation of fungi from the pap samples revealed that it could serve as a means for the transmission of potentially pathogenic microorganisms. However, fungi isolates such *Rhizopus sp.* and *Aspergillus sp.* was not present in pap due to the fact that they are mesophilic fungi and cannot survive at temperature ranges above 60°C. Comparison of samples showed that pap preparation is an efficient technique for elimination of fungi but significantly ineffective in complete elimination of bacteria. It can therefore be concluded that cooking of pap does not entirely eliminate pathogenic microbes and hence caution should be taken in pap consumption

**Key words: Isolation, Identification, Microorganisms, Associated, Pap, Stored, Corn, Starch.**

## Introduction

Maize is a crop that is extensively consumed throughout the majority of Africa and the world at large, playing a crucial role in the economies and cultures of the people who grow it. Due to its inherent nutritional value, it is recognized as a key raw material in the human diet and is cultivated worldwide as an annual cereal crop (Amakoromo, 2011).

Maize is used in Nigeria to make a variety of nutritious products. It can be cooked into a smooth gel or combined with hot water to create pap, a porridge-like food. A healthy meal, papaya is high in carbohydrates, natural antioxidants, vitamins A and C, potassium, chromium, selenium, zinc, phosphorus, magnesium, and many other nutrients. It is also a strong source of B vitamins, including pantothenic acid (B5), niacin (B3), riboflavin, and thiamine (B1). Nevertheless, there aren't any sizable factories producing pap at the moment. Rather, in many regions of the nation, the production of papaya is still a vital home-based business that is run on a modest scale by some housewives for profit (Eromosele et al., 2020).

Research has revealed that microbiological contamination can occur in maize flour, or maize product, when it is processed into pap. This is a result of the preparation procedure, conventional fermentation, and malting methods, which just do not ensure high standards of cleanliness and are therefore vulnerable to microbial contamination. In actuality, the traditional fermentation methods used to make papa are typically unplanned and spontaneous (Adegunwa and Alamu, 2011).

Pap's pH 4.8 acidity is known to prevent the growth of certain germs. These microbes may produce hazardous compounds and are important contributors to the pap rotting process. Other extrinsic variables, including storage, can contribute to the spoiling of papaya (Ogodo et al., 2015). The microbiology of pap and its related products has not received much attention from African scholars. Instead, the use of starter cultures, which use bacteriogenic lactic acid bacteria (LAB) to treat and prevent a variety of water-borne diseases, is receiving more attention these days.

The purpose of evaluating the microbiological quality of pap is to find the pollutants linked to incorrect storage over a reasonable amount of time. Inadequate storage can give rise to additional contaminants that pose a risk to consumers, particularly kids. This can result in food poisoning or

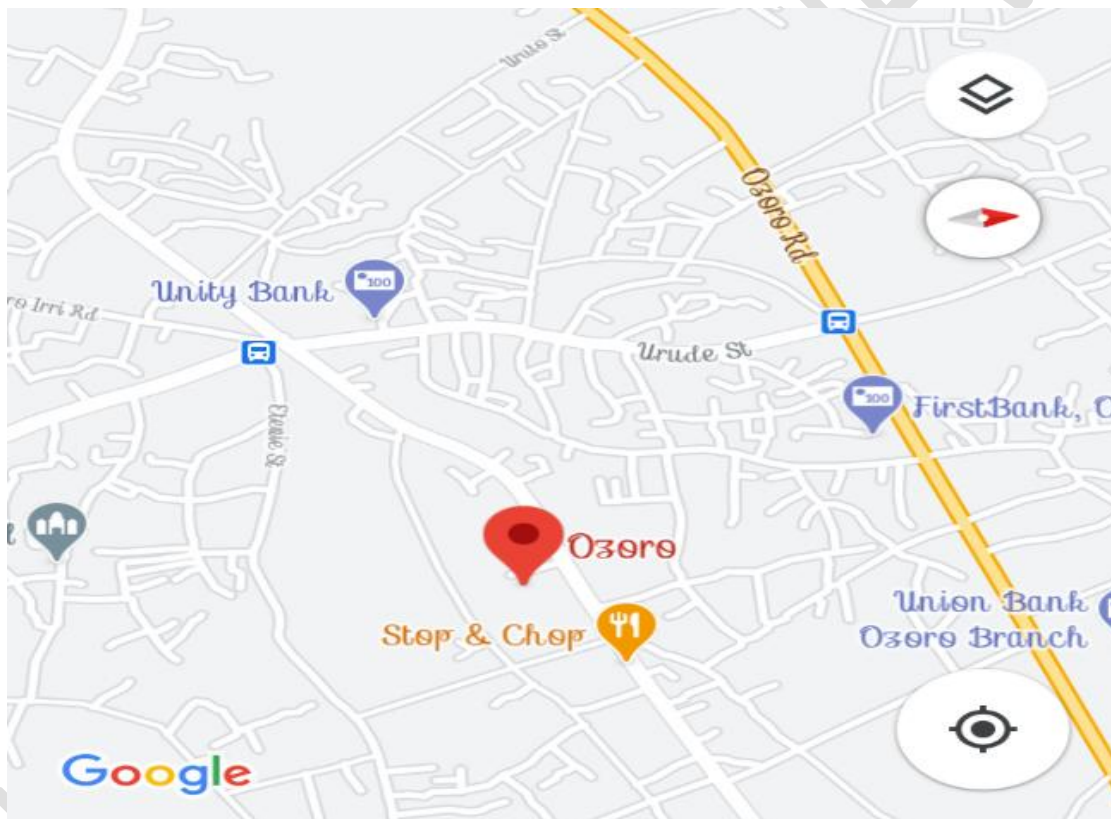
alcohol intoxication. In addition to storage, which leads to a growth in microorganisms, Iyare et al. (2020) claimed that there may be health risks. As a result, it is vital to assess the food safety of the stored flour and the pap made from maize flour.

UNDER PEER REVIEW

## Materials and Methods

### Study Area

Ozoro is the headquarters of Isoko North Local Government Area of Delta State. It is one of the administrative units of the Isoko regions in Delta State Nigeria situated at Latitude: 5.5383 and Longitude:6.2161 with approximate population of 13,411(at 2015) inhabitants and land mass of 1.136km<sup>2</sup>. It is home to anthropogenic activities associated with the production of pap which is sold both locally by hawking and commercially in its local sited market environment.



Map 1 :Map showing study location

### Collection of Sample/Preparation

Raw pap was divided into 2 portions, and properly labeled as sample 1 and sample 2. The samples were soaked with water in a bowl for seven (7) days. Sample 1 was prepared daily using warm water and also with a constant change of water while, sample 2 was left for 7days without constant change of water.

## Materials

For the purpose of this research the following and materials were used to culturing of bacteria and also for experimental analysis.

Table 1: Materials used for the Assessment

| <b>Material</b>        | <b>Manufacturer</b> |
|------------------------|---------------------|
| Measuring Cylinder     | Pyrex               |
| Beaker                 | Pyrex               |
| Conical flask          | Pyrex               |
| Distilled Water Holder | Pyrex               |
| Stirring Rod           | Pyrex               |
| Test Tube              | Pyrex               |
| Maize Sample           |                     |
| Sampling Container     |                     |
| Petri Dish             |                     |

Table 2: Reagents used for the Assessment

| <b>Material</b>         | <b>Manufacturer</b> |
|-------------------------|---------------------|
| Acetone                 |                     |
| Nutrient Agar           |                     |
| Sabourand Dextrose Agar |                     |
| Peptone Water           |                     |
| Crystal Violet          |                     |

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Lugol's Iodine

Tryptophan Broth Sampling Container

Distilled water

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### **Media Preparation and Serial Dilution**

7g of nutrient agar and Sabouraud agar dextrose were mixed with distilled water and heated with an autoclave. Nutrient agar was used for the isolation of bacteria while agar (SDA) was used for the isolation of fungi.

For serial dilution, ten-fold serial dilution was carried out using sterile distilled water as the diluents. 10 test tubes containing ten milliliter volume per volume (10mlv/v) of sterile distilled water was used for each of the sample. The samples were properly labeled using numerals (1–10) and arranged appropriately in a test tube rack. 10ml of the sample was serially diluted in test tubes containing 10ml of distilled water using a sterile syringe and each transfer followed by a gentle agitation in order to mix the contents uniformly. Same procedure was repeated for all the diluents in the same manner. The serial dilution was performed aseptically beside a lit bunsen burner to prevent contamination.

### **Determination of Total Heterotrophic Bacteria (THB) and Total Heterotrophic Fungi (THF)**

Microbial evaluation was determined using APHA 9215C spread plate method. 1.0g of the sample was weighed and diluted with 9ml of sterilized water in sterilized test tubes to make serial dilutions of  $10^{-3}$  to  $10^{-8}$  for total heterotrophic bacteria. 0.1ml aliquot was taken from one of the serial dilutions and poured on already prepared nutrient agar plates. Plates were hence incubated at  $37^{\circ}\text{C}$  for 24hours and then the colonies on the plate were enumerated using a colony

counter. For total heterotrophic fungi, 0.1ml aliquot was taken from one of the serial dilutions and poured on already prepared extract agar plates. Plates were hence incubated at  $28\pm 2^{\circ}\text{C}$  for 3-5 days and then the colonies on the plate were enumerated using a colony counter.

### **Bacteria Identification**

Identification of the isolates using phenotypic characteristics was based on the various test carried out using Bergey's manual of systemic.

### **Gram Staining**

Smear of bacteria culture was made on clean glass slide, air dried and heated. Smear was covered with crystal violet for 30seconds, washed with distilled water and covered with iodine solution for 60seconds. Slide was again washed with 95% ethyl alcohol and distilled water. Again the smear was covered with safranin for 30seconds, washed with distilled water and blot dried. The air dried slide was then viewed under the microscope.

### **Motility Test**

Sterilized wire loop was used to make a drop of the test organism on a clean slide. Three drops of peptone water was then added and the slide was covered with a slip and examined microscopically under 45X objective.

### **Catalase Test**

Nutrient agar medium was prepared and poured into culture tubes and flasks before sterilization. The agar slants were then incubated with organisms and an inoculated slant was kept as a control. The cultures were incubated at  $35^{\circ}\text{C}$  and 3-4 drops of the hydrogen peroxide was added on the growth of each slant culture. The culture was then observed for the appearance or absence of gas bubbles. Active bubbling shows positive catalase and absence shows negative catalase.

### **Oxidase Test**

A piece of filter paper was divided into three equal sections and labeled with the name of organisms. A loop full of the culture was rubbed on the moistened filter paper using a sterile loop. The colour of the smear was checked exactly 15-30 seconds after rubbing the cell on the reagent moistened filter paper. A deep blue color indicates positive reaction. Light violet or purple color which developed within 10 seconds was recorded.

### **Indole Production Test**

1% tryptone broth was prepared and sterilized using autoclave at 151bs for 15 minutes. The tryptone broth was inoculated with test organism and an uninoculated tube was kept as control. The tubes were inoculated at 35°C for 48 hours; 1ml of Kovac's reagent was added after 48 hours of inoculation. The tubes were then shaken at intervals of 10 minutes and allowed to stand to permit the reagent to come to the top. The tubes were then observed for cherry red layers with red surfaces indicating positive indole.

### **Starch Hydrolysis Test**

Starch agar was prepared and sterilized using an autoclave at 151bs for 15 minutes. The medium was poured into a petri plate and allowed to solidify and test organisms were inoculated on to the plate with a sterile loop. The plates were then incubated at 35°C for 48 hours, flooded with Gram's iodine and observed for clear zone around the test organism.

### **Hydrogen Sulphide Test**

SIM agar was prepared and sterilized using an autoclave. The agar tubes were then labeled with the name of the organisms to be inoculated and inoculated appropriately. The tubes were then incubated at 35°C to 36°C for 48 hours and observed for the presence of black coloration along the line of inoculation.

### **Coagulate Test**

A drop of distilled water was placed on two slides and colony of test organism emulsified on each slide to make a thick suspension. A loopful of plasma was then added to one of the suspension and mixed gently. This was observed for clumping of the organism within 10seconds.

Clumping of the organism indicated positive reaction.

### **Citrus Utilization Test**

Simmon's citrate agar medium was prepared and sterilized using an autoclave. 5ml of media was hence poured into the culture tube and agar slants were prepared and inoculated with test organisms. The uninoculated tubes were kept as control and all tubes incubated at 37°C for 48hours. Slant cultures were hence observed for growth and coloration of the media.

### **Methyl-Red**

MRVP broth was prepared and sterilized using an autoclave and 5ml of broth poured into separate tubes. Tubes were then inoculated with test organisms and incubated at 25°C for 48hours. 5 drops of methylred indicator were added to the tubes of each set and change in colour was observed for MR test.

### **Fungi Identification**

Sufficient fungi growth was observed after which the colony were covered with 5ml of 0.0% sterile saline. Suspensions were then made gently by probing the surface of the covered colonies with the tip of a sterile pasteur pipette. The suspension were immediately transferred to a sterile tube and allowed to settle for 15minutes at room temperature after which the homogenous upper liquid was decanted and used for further experiment. For the identification, both microscopic and macroscopic features of the hyphal mass, morphology of the produced spore and the nature of the fruiting bodies were considered.

## Results and Discussions

### Results

Results from laboratory analysis of cooked, Raw and Steeped pap are represented below;

Table 3: Result of Cooked, Raw and Steeped Pap over a Retention time of Seven Days

| Day                   | Raw                         |                             | Cooked                      |                             |
|-----------------------|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
|                       | THB x 10 <sup>6</sup> cfu/g | THF x 10 <sup>2</sup> cfu/g | THB x 10 <sup>6</sup> cfu/g | THF x 10 <sup>2</sup> cfu/g |
| 1                     | 6.1                         | 0.5                         | 1.1                         | ND                          |
| 2                     | 8.4                         | 0.6                         | 1.4                         | ND                          |
| 3                     | 9.8                         | 0.8                         | 1.8                         | ND                          |
| 4                     | 11.3                        | 0.9                         | 2.2                         | ND                          |
| 5                     | 12.1                        | 0.9                         | 2.6                         | ND                          |
| 6                     | 13.4                        | 1.2                         | 2.4                         | ND                          |
| 7                     | 15.8                        | 1.6                         | 3.1                         | ND                          |
| <b>7 days Steeped</b> | 27.5                        | 2.6                         | 4.3                         | ND                          |

Note: THB = Total Heterotrophic Bacteria, THF = Total Heterotrophic Fungi

Table 4a: Result of Bacteria Isolated on Cooked, Raw and Steeped Pap

| Days   | Samples | Morphological Test |       | Biochemical Test |          |           |         |       |                   |                             |    |    | Species Name             |
|--------|---------|--------------------|-------|------------------|----------|-----------|---------|-------|-------------------|-----------------------------|----|----|--------------------------|
|        |         | Gram Stain         | Shape | Motility         | Catalase | Coagulase | Oxidase | Spore | Indole Production | H <sub>2</sub> S Production | CU | SH |                          |
| 1      | Raw     | -                  | Rod   | -                | +        | -         | +       | -     | -                 | -                           | -  | -  | <i>Pseudomonas sp.</i>   |
|        | Cooked  | -                  | Rod   | -                | +        | -         | +       | -     | -                 | -                           | -  | -  | <i>Pseudomonas sp.</i>   |
| 2      | Raw     | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
|        | Cooked  | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
| 3      | Raw     | +                  | Rod   | -                | -        | -         | -       | -     | -                 | -                           | -  | -  | <i>Lactobacillus sp.</i> |
|        | Cooked  | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
| 4      | Raw     | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
|        | Cooked  | +                  | Rod   | -                | -        | -         | -       | -     | -                 | -                           | -  | -  | <i>Lactobacillus sp.</i> |
| 5      | Raw     | -                  | Rod   | -                | +        | -         | +       | -     | -                 | -                           | -  | -  | <i>Pseudomonas sp.</i>   |
|        | Cooked  | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
| 6      | Raw     | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
|        | Cooked  | -                  | Rod   | -                | +        | -         | +       | -     | -                 | -                           | -  | -  | <i>Pseudomonas sp.</i>   |
| 7      | Raw     | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
|        | Cooked  | +                  | Rod   | -                | -        | -         | -       | -     | -                 | -                           | -  | -  | <i>Lactobacillus sp.</i> |
| 7 days | Raw     | +                  | Rod   | +                | +        | -         | -       | +     | -                 | -                           | -  | +  | <i>Bacillus sp.</i>      |
|        | Cooked  | +                  | Rod   | -                | -        | -         | -       | -     | -                 | -                           | -  | -  | <i>Lactobacillus sp.</i> |

Note: CU = Citrate utilization, SH = starch hydrolysis

Table 4b: Result of Fungi Isolated on Cooked, Raw and Steeped Pap

| Days      | Sample | Shape | Surface         | Elevation | Spore colour | septation   | Reproduction | Probably Organism                               |
|-----------|--------|-------|-----------------|-----------|--------------|-------------|--------------|---|
| 1         | Raw    | C     | Powdery         | SR        | Brown        | Septate     | Sexual       | <i>Aspergillus sp.</i>                          |
| 2         | Raw    | C     | Powdery         | SR        | Army brown   | Septate     | Sexual       | <i>Aspergillus sp.</i>                          |
| 3         | Raw    | C     | Powdery         | SR        | brown        | Septate     | Sexual       | <i>Aspergillus sp.</i>                          |
| 4         | Raw    | C     | cottony         | R         | white        | Non Septate | both         | <i>Rhizopus sp.</i>                             |
| 5         | Raw    | C     | cottony         | R         | white        | Non Septate | both         | <i>Rhizopus sp.</i>                             |
| 6         | Raw    | C     | cottony         | R         | white        | Non Septate | both         | <i>Rhizopus sp.</i>                             |
| 7         | Raw    | C     | cottony         | R         | white        | Non Septate | both         | <i>Rhizopus sp.</i>                             |
| 7 (steep) | Raw    | C     | Cottony/powdery | R         | White/brown  | Non Septate | both         | <i>Rhizopus sp.</i> ,<br><i>Aspergillus sp.</i> |

NOTE: SM = semi raised, R = raised and C = circular

## Discussion

### Bacteriological Result of Raw and Cooked Pap

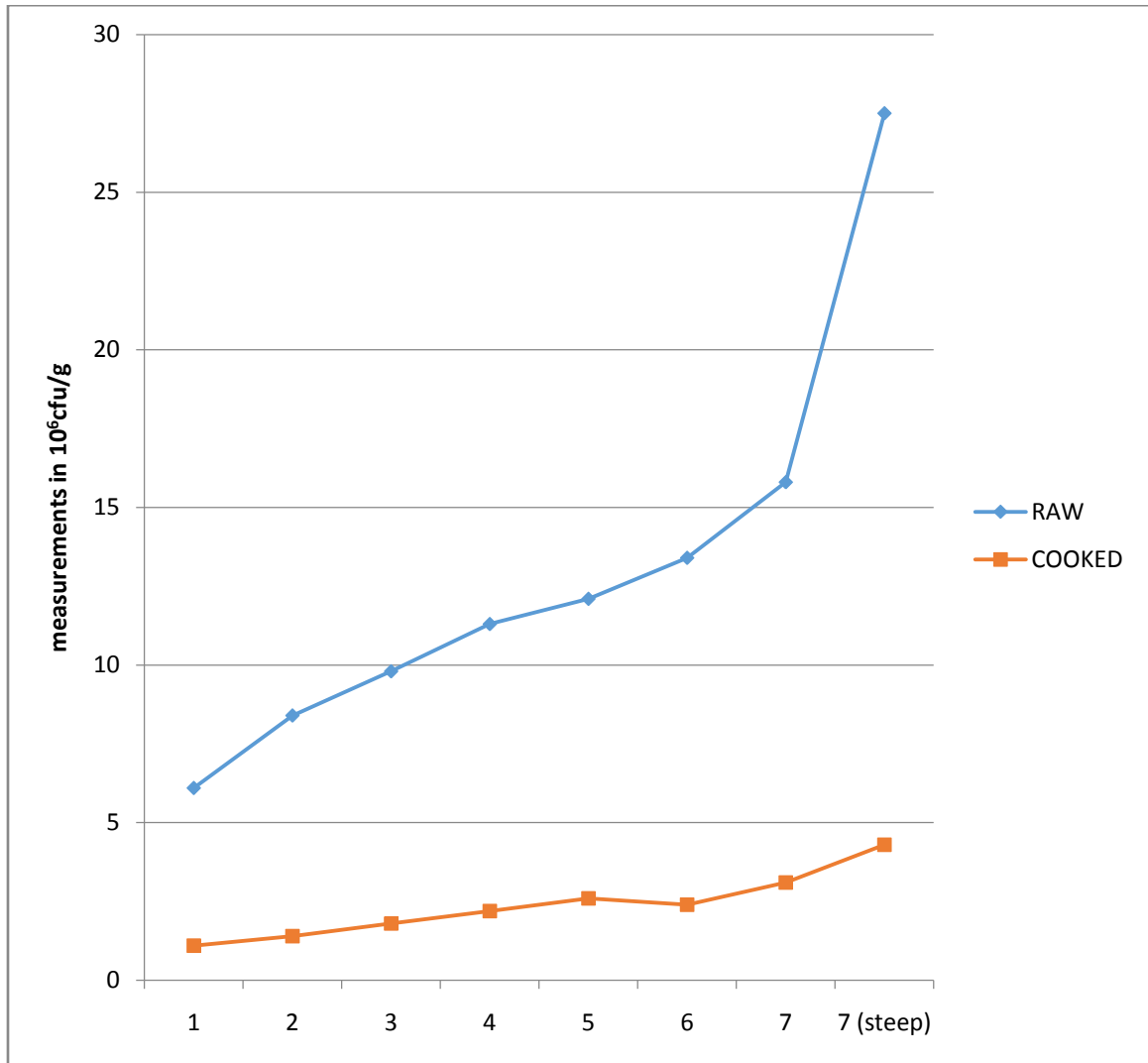


Figure 1: Total Heterotrophic Bacteria Result of Raw and Cooked Pap Sample

Result obtained shows that the pap samples are contaminated with bacteria. According to Omemu and Adeosun (2010) this contaminated could have been as a result of unhygienic practice during pap preparation and also through the muslin clothes used in sieving the shaft due to lack of sterilization.

The profile of bacteria evaluated shows an increasing trend over the retention period as shown in figure 1. This trend increases for both raw and cooked pap and this can be attributed to

spontaneous fermentation which may have occurred especially in raw sample. Bacteria growth was observed to be low between the first to second day ( $6.1 - 8.4 \times 10^6$  cfu/g) but extended largely at the seventh day. This can be attributed to adaptation period (lag phase), in which bacteria try to adapt to the existing environment. Study by Rahmawati *et al.*, (2013) on isolation and identification of microorganisms during spontaneous fermentation of maize reported a similar increasing trend from 4cfu/mL to 6cfu/mL after a retention time of 72hours. This is study also confirmed result obtained by Onyezeet *al.*, (2013) which revealed that the bacteria isolated from both steep water and soaked pap were *Lactobacillus sp.* and *Leuconostocsp.*. Observed result from cooked pap indicated that the bacteria are not entirely eliminated when prepared in heat and this can be due to the strain of microbe (*Pseudomonas sp.*, *Bacillus sp.* and *Lactobacillus sp.*) present which is identified to survive a temperature range between  $50 - 80^{\circ}\text{C}$  for five minutes for *pseudomonas sp.*,  $95 - 135^{\circ}\text{C}$  for *Bacillus sp.* and *Lactobacillus sp.* therefore this is the reason for the dominant nature of *Bacillus sp.* and its high tolerance ability. This is also because pap preparation is often done at a reduced time range. The overall result observed after seven days retention time for raw pap ( $15.8 \times 10^6$  cfu/g) was lower than observed values of steep pap ( $27.5 \times 10^6$  cfu/g) which shows that daily changing of water for raw pap is also an effective technique for bacteria reduction. Hence, consumption of pap can served as transmission medium of potentially pathogenic microorganisms and present significant health risk. The occurrence of these pathogens in pap suggests the need for caution in the use of these foods in infant feeding.

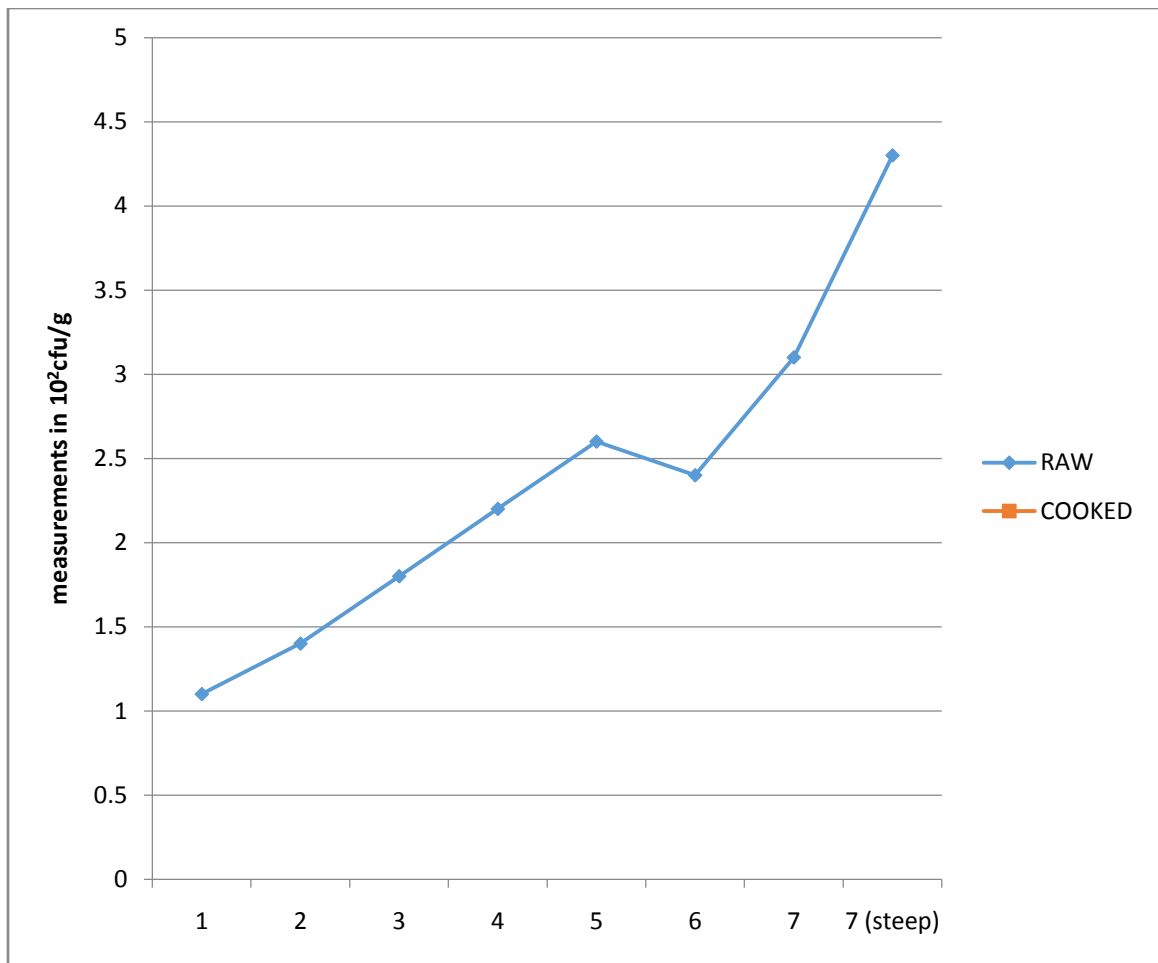


Figure 2: Total Heterotrophic Fungi Result of Raw and Cooked Pap Sample

The isolation of fungi from the pap samples revealed that it could serve as a means for the transmission of potentially pathogenic microorganisms. From figure 2, it was observed that the load of fungi increased overtime and both varied significantly from steep pap sample which was relatively higher. The observed fungi isolated were *Rhizopus sp.* and *Aspergillus sp.* which were similarly observed by Iyaraet *al.*, (2020) and can be attributed to possible pap contamination. Result from table 4a also showed that cooking of pap was a significantly efficient technique to elimination of fungi from pap as no fungi growth was observed in cooked pap sample. This can be attributed to the fact that *Rhizopus sp.* and *Aspergillus sp.* are mesophilic fungi and cannot survive at temperature ranges above 60°C. However, this study shows no health risk from fungi contamination.

## **Conclusion and Recommendations**

### **Conclusion**

From result observed and reported in table 3, it can be stated that pap could pose a threat to health of consumer based on the types and numbers of pathogenic microorganisms isolated from the raw and cooked samples. The observed result portrays the presence of *Bacillus sp.*, *Lactobacillus sp.*, *Pseudomonas sp.*, *Rhizopus sp.* and *Aspergillus sp.* these microorganisms varied in concentrations across the retention time (in days). Comparison of samples showed that pap preparation is an efficient technique for elimination of fungi but significantly ineffective in complete elimination of bacteria. It can therefore be concluded that cooking of pap those not entirely eliminate pathogenic microbes and hence caution should be taken in pap consumption.

### **Recommendations**

From the above conclusion, the following recommendations are made;

- I. Hygienic practices should be incorporated to prevent pap consumption since its negligence leads to possible contamination.
- II. Pap producing materials should be sterilized regularly before use.
- III. Pap should be prepared in cooking pans and subjected to a minimum of 10 minutes cooking time to ensure complete elimination of bacteria.

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