South Asian Journal of Research in Microbiology

Volume 18, Issue 6, Page 1-9, 2024; Article no.SAJRM.115982
ISSN: 2582-1989

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

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/SAJRM/2024/v18i6363

Open Peer Review History:
This journal follows the Advanced Open Peer Review policy. Identity of the Reviewers, Editor(s) and additional Reviewers, peer review comments, different versions of the manuscript, comments of the editors, etc are available here: https://www.sdiarticle5.com/review-history/115982

Original Research Article

Received: 15/02/2024
Accepted: 17/04/2024
Published: 08/05/2024

ABSTRACT

This study isolated and identified 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 sp* and *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^{O}C. Comparison of samples showed that

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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.

Keywords: Associated; corn; identification; isolation; microorganisms; pap; starch; stored.

1. 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 [1]. Study by Ulrah et al. [2] showed that maize the mineral content of maize as sodium (540ppm), potassium (2915ppm) and Calcium (410ppm). Other constituents include fats 3-7.2%, crude fiber 0.8-2% and energy 307kcal/100g.The protein contents and other components of the grain may vary depending on the genotype and the environment. Uarrota et al. [3], working with eight landrace varieties of maize, found protein levels ranging from 7.04 to 11.59 g/100 g of grains, while lipids ranged from 3.01 to 5.53g. Enyisi et al. [4] also reported the proximate composition of maize and maize products in Nigeria the range of 11.6- 20.0% (moisture), 1.10 – 2.95% (Ash), 4.50 – 9.87% (protein), 2.17-4.43 (fat), 2.10- 26.70% (fibre) and 44.60- 69.60% (carbohydrate).

Basically, in Nigeria, the most common source of maize source is through the consumption of maize flour which is prepared by draining, grinding drying and spontaneous fermentation [5]. According to Awaneesh [6] maize can be divided into eight groups on the basis of the endosperm of kernels including dent maize, flint maize, pop maize, baby maize, sweet amongst others. 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 [7].

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 [8].

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 [9]. 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. [10] 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.

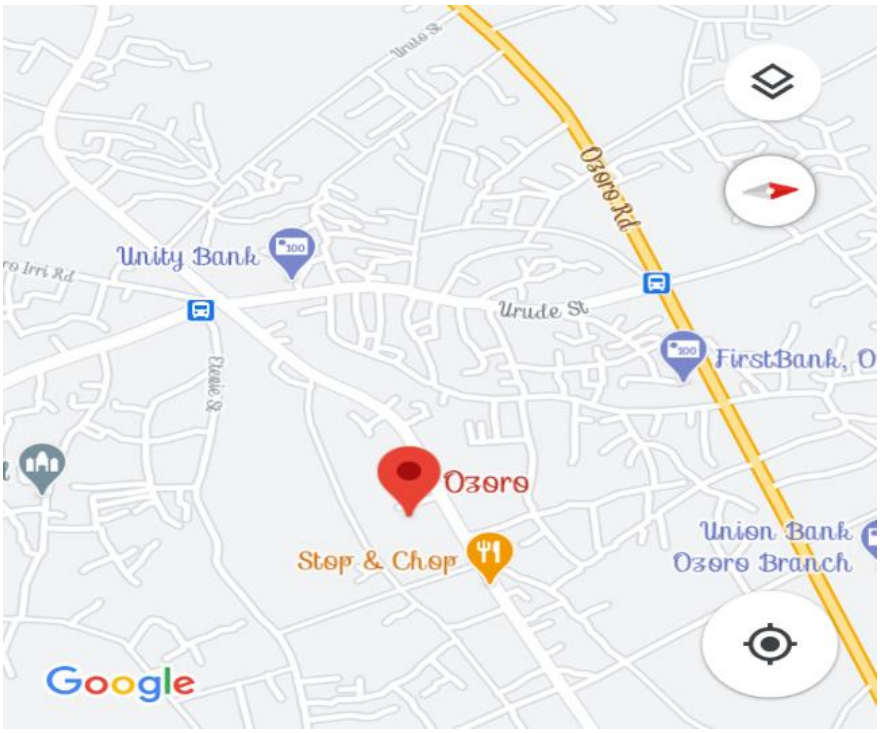
2. MATERIALS And METHODS

2.1 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^2. 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.

2.2 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.



Map 1. Map showing study location

2.3 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 (10mllv/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 other 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.

2.4 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^{O}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 ± 2^{O}C for 3-5days and then the colonies on the plate were enumerated using a colony counter.

2.5 Bacteria Identification

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

2.6 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 30secnds, washed with distilled water and blot dried. The air dried slide was then viewed under the microscope.

2.7 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.

2.8 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 35OC 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.

2.9 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 moisture filter paper using a sterile loop. The colour of the smear was checked exactly 15-30seconds 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.

2.10 Indole Production Test

1% tryptone broth was prepared and sterilized using autoclave at 151bs for 15 minutes. The trytone broth was inoculated with test organism and an uninoculated tube was kept as control. The tubes were inoculated at 35OC for 48hours; 1ml of Kovac s reagent was added after 48hours 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 rod layers with red surfaces indicating positive indole.

2.11 Starch Hydrolysis Test

Starch agar was prepared and sterilized using an autoclave at 151bs for 15minutes. 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 $35^{O}C$ for 48hours, flooded with grams iodine and observed for clear zone around the test organism.

2.12 Hydrogen Sulphide Test

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

2.13 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.

2.14 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 $37^{O}C$ for 48hours. Slant cultures were hence observed for growth and coloration of the media.

2.15 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 25Oc for 48hours. 5 drops of methyl red indicator were added to the tubes of each set and change in colour was observed for MR test.

2.16 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 pastcur pipette. The

suspension were immediately transferred to a sterile tube and allowed to settle for 15 minutes 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.

3. RESULTS AND DISCUSSION

3.1 Results

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

Table 1. Result of Cooked, Raw and Steeped Pap over a Retention time of Seven Days

Day	Raw		Cooked	
	THB x 10^6cfu/g	THF x 10^2cfu/g	THB x 10^6cfu/g	THF x 10^2cfu/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
7 days Steeped	27.5	2.6	4.3	ND

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

Table 2. 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_2S Production	CU	SH	
1	Raw	-	Rod	-	+	-	+	-	-	-	-	-	*Pseudomonas sp.*
	Cooked	-	Rod	-	+	-	+	-	-	-	-	-	*Pseudomonas sp.*
2	Raw	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
	Cooked	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
3	Raw	+	Rod	-	-	-	-	-	-	-	-	-	*Lactobacillus sp.*
	Cooked	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
4	Raw	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
	Cooked	+	Rod	-	-	-	-	-	-	-	-	-	*Lactobacillus sp.*
5	Raw	-	Rod	-	+	-	+	-	-	-	-	-	*Pseudomonas sp.*
	Cooked	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
6	Raw	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
	Cooked	-	Rod	-	+	-	+	-	-	-	-	-	*Pseudomonas sp.*
7	Raw	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
	Cooked	+	Rod	-	-	-	-	-	-	-	-	-	*Lactobacillus sp.*
7 days	Raw	+	Rod	+	+	-	-	+	-	-	-	+	*Bacillus sp.*
	Cooked	+	Rod	-	-	-	-	-	-	-	-	-	*Lactobacillus sp.*

Note: CU = Citrate utilization, SH = starch hydrolysis

Table 3. 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	*Aspergillus sp.*
2	Raw	C	Powdery	SR	Army brown	Septate	Sexual	*Aspergillus sp.*
3	Raw	C	Powdery	SR	brown	Septate	Sexual	*Aspergillus sp.*
4	Raw	C	cottony	R	white	Non Septate	both	*Rhizopus sp.*
5	Raw	C	cottony	R	white	Non Septate	both	*Rhizopus sp.*
6	Raw	C	cottony	R	white	Non Septate	both	*Rhizopus sp.*
7	Raw	C	cottony	R	white	Non Septate	both	*Rhizopus sp.*
7 (steep)	Raw	C	Cottony/powdery	R	White/brown	Non Septate	both	*Rhizopus sp., Aspergillus sp.*

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

3.2 Bacteriological Result of Raw and Cooked Pap

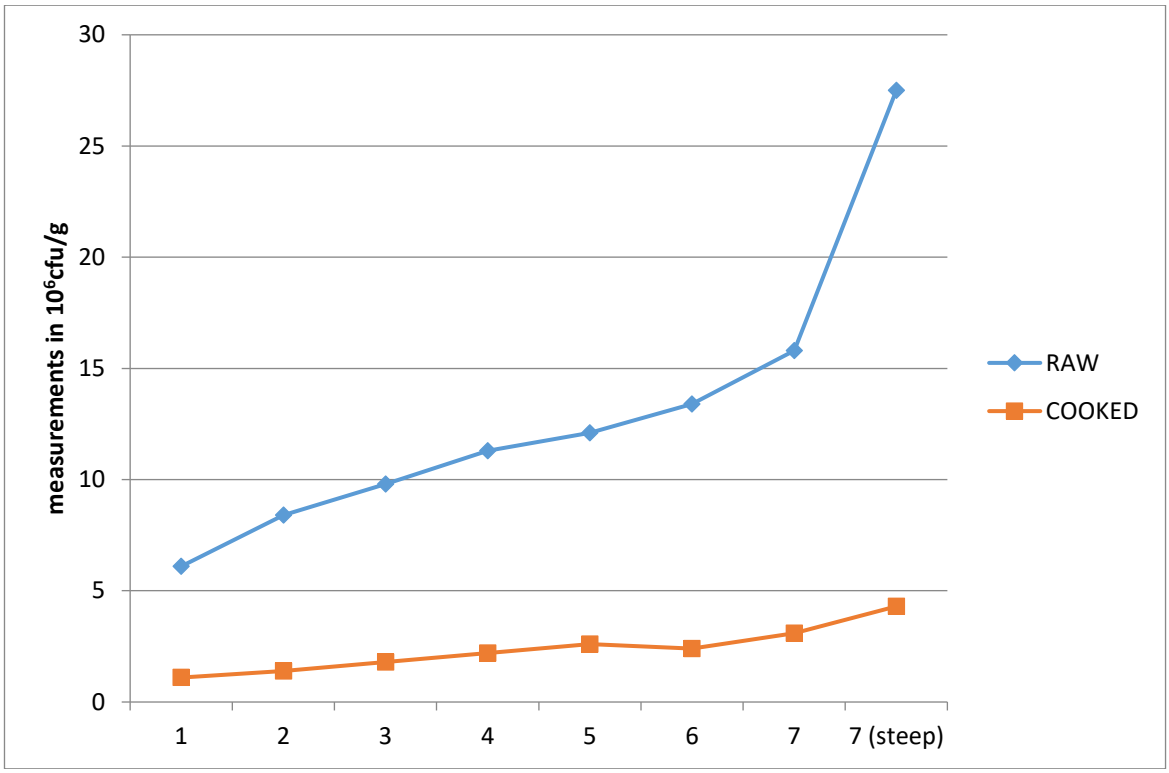


Fig. 1. Total Heterotrophic Bacteria Result of Raw and Cooked Pap Sample

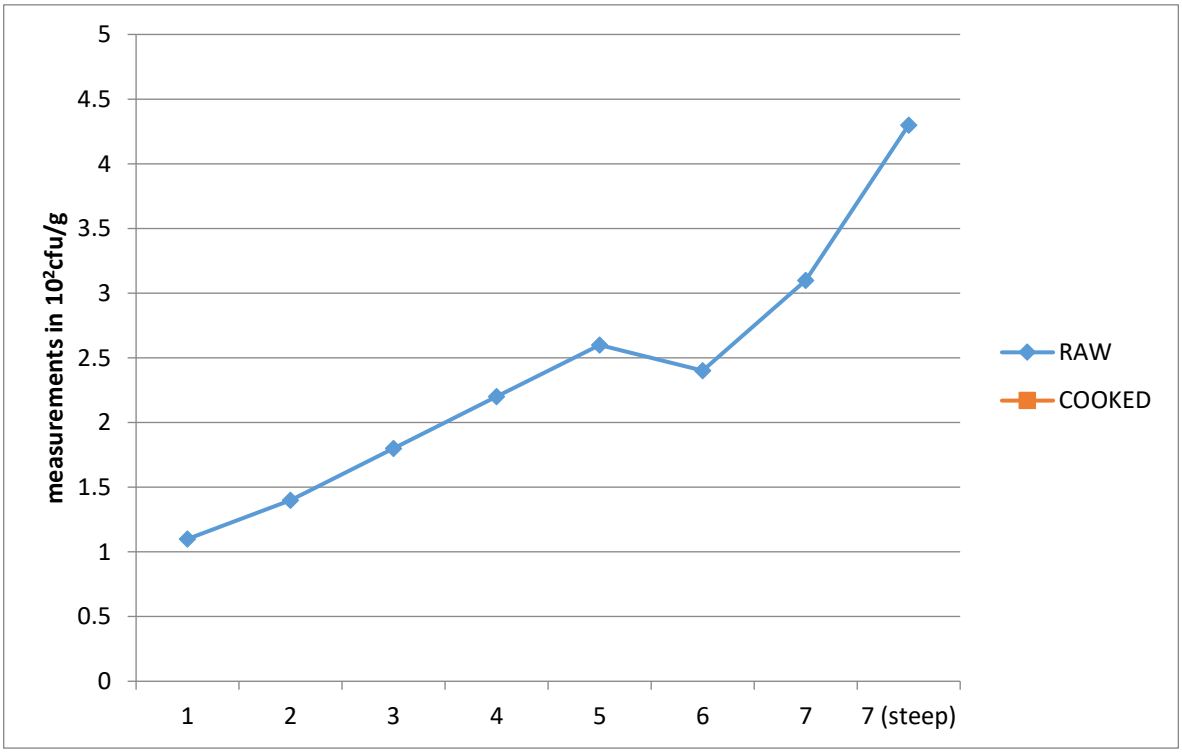


Fig. 2. Total Heterotrophic Fungi Result of Raw and Cooked Pap Sample

3.3 Discussion

Result obtained shows that the pap samples are contaminated with bacteria. According to Omemu and Adeosun [11] 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 Fig. 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 – 8.4x10^6cfu/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. [12] 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 Onyeze et al. [13] which revealed that the bacteria isolated from both steep water and soaked pap were *Lactobacillus sp.* and *Leuconostoc sp..* 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^{O}C for five minutes for *pseudomonas sp.*, 95 – 135^{O}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 x 10^6cfu/g) was lower than observed values of steep pap (27.5 x 10^6cfu/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.

The isolation of fungi from the pap samples revealed that it could serve as a means for the transmission of potentially pathogenic microorganisms. From Fig. 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 Iyara *et al.,* (2020) and can be attributed to possible pap contamination. Result from Table 1 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^{O}C. However, this study shows no health risk from fungi contamination.

4. CONCLUSION AND RECOMMENDATIONS

From result observed and reported in Table 1, 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.

From the above conclusion, the following recommendations are made; Hygienic practices should be incorporated to prevent pap consumption since its negligence leads to possible contamination.

Pap producing materials should be sterilized regularly before use. Pap should be prepared in cooking pans and subjected to a minimum of 10munites cooking time to ensure complete elimination of bacteria.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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Peer-review history:
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