



## Evaluation of the Microbial Load and pH of Pap Produced from Selected Cereals

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### Authors' contributions

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

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### ABSTRACT

The aim of this work was to evaluate the microbial load of pap samples made from three commonly utilized cereal grains namely: maize (*Zea mays*), millet (*Pennisetum typhoideum*) and guinea corn (*Sorghum bicolor*). Exactly 2 kg each of the aforementioned grains was steeped in 1000 ml of clean water for 3 days at  $30\pm 2^{\circ}\text{C}$  temperature. The water was disposed and the cereal grain separately washed thoroughly using clean water. The grains were then wet milled and the resulting paste mixed with 1000 ml of clean water. The slurry was filtered with the aid of the muslin cloth. The filtrate was then allowed to stand for a period of 24 hours and the supernatant disposed. Microbiological analysis to determine microbial load on the various samples were carried using standard procedures. Guinea corn based pap had the highest lactic acid bacteria count of  $9\times 10^3$  CFU/g at 0 hr of fermentation which decreased to  $6.6\times 10^5$  CFU/g at the 72<sup>nd</sup> hr. On the other hand, the composite of the three cereals had the lactic acid bacteria count of  $4.2\times 10^4$  CFU/g at 0 hr of fermentation which increased to  $8.6\times 10^5$  CFU/g at the 72<sup>nd</sup> hr, the highest value recorded for all samples studied. Total coliform count on all samples maintained a uniform trend of increase at the

24<sup>th</sup> and 48<sup>th</sup> hr and a decrease at the 72<sup>nd</sup> hr. Millet based pap had the highest fungi count of  $2.3 \times 10^1$  CFU/g at 0hr of fermentation which increased progressively to  $4.9 \times 10^1$  at the 72<sup>nd</sup> hr. However, pap derived from the composite of the cereals presented the lowest fungi count of  $0.52 \times 10^2$  at 0hr which however increased progressively to record the highest count of  $1.4 \times 10^1$  at the 72<sup>nd</sup> hr. In conclusion, there is need to optimize the growth of lactic acid bacteria in the production of pap, this may enhance its acid production potential and consequent inhibition and/destruction of potential harmful microorganisms in fermented foods.

**Keywords:** Cereals; fermentation; microbial load; pap.

## 1. INTRODUCTION

Pap popularly called ogi, akamu and akassan among the Yoruba, Igbo and Hausa speaking peoples of the Western, Eastern and Northern Nigeria respectively [1] is a fermented non-alcoholic starchy food mainly consumed in the Southern part of Nigeria. It is a sour fine paste beverage which when cooked produces a thin, semi-solid porridge [2]. It is the primary native food used in weaning babies to supplement breast milk and the breakfast for the pre-school children adults. Pap is also a recommended meal for convalescing patients owing to its high digestibility [3].

Cereals are the most important source of the world's food which have significant impact in human diet throughout the world [4]. Cereal grains form an important group of substrate for fermented foods in tropical Africa [5]. Aside rice, other main cereals grown in Nigeria include maize (*Zea mays*), millet (*Pennisetum typhoideum*) and guinea corn (*Sorghum bicolor*) all of which are ideal substrate for the production of pap [6]. Traditional preparation of pap generally entails soaking or steeping of a cereal in warm water for 2-3 days followed by wet milling and sieving with a screen mesh to get rid of the bran, hulls, and germs [7], while the filtrate is fermented for 2-3 days to yield pap, a sour, white, starchy sediment [3]. Fermentation is a process that helps to break down large organic molecules via the action of microorganisms into simpler ones. The microbial or enzymatic actions on food ingredients tend to ferment food, leading to desirable biochemical changes responsible for the significant modifications in food. It is a natural way of improving the nutritional quality as well as acceptability of foods [8]. Fermentation increases the acidity of a fermented food product to preserve it from microorganisms which are known to have pH optimum, minimum and maximum for growth in food [9]. Thus, extensive information on the acidity and microbial load of the freshly produced pap can be explored for better preservation [3].

## 2. MATERIALS AND METHODS

### 2.1 Collection of Cereals

Cereals used in this work namely; Maize (*Zea mays*), Millet (*Pennisetum vulgare*) Guinea corn (*Sorghum bicolor*), were obtained from Nkwoabuo market in Uturu, Isukwuako Local Government Area of Abia State. Dirt was carefully removed from the said grains before use.

### 2.2 Pap Production

A known weight (2 kg) each of maize, millet and guinea corn was soaked in 1000 ml of clean water for 3 days at room temperature ( $30 \pm 2^\circ\text{C}$ ). The water was discarded and the cereal grain separately and thoroughly washed with clean water. The wet grains were milled using attrition mill and the resulting paste mixed with 1000 ml of clean water. The slurry of the mixture was filtered with the aid of a muslin cloth. The filtrate was then allowed to stand for 24 hours and the supernatant was disposed. The resulting fresh pap was placed inside a muslin cloth and allowed to stand for the next 12hr so as to allow more water to drain off [10].

### 2.3 Determination of pH

The pH of the pap samples obtained from the three different cereals was determined in accordance with the method of AOAC [11]. A known weight 10 g each of the samples was mixed in 100 ml of  $\text{CO}_2$  - free distilled water. The mixture was allowed to stand for 15 mins shaken at 5 mins interval and filtered with Whatman No. 14 filter paper. The pH of the filtrate was measured in triplicate using a pH meter.

### 2.4 Microbiological Analysis

Exactly 10 g each of the freshly fermented pap samples was homogenized in 90 ml of sterile distilled water for 30 s. The mixture was serially

diluted in sterile distilled water by the method of Meynell and Meynell [12] and from the 10 fold dilutions, colony-forming units (cfu) were determined using pour plate method. Plate counts were carried out using the following media, temperature and incubation periods; De Mann Rogosa-Sharpe (MRS) agar 37°C, 48 h for LAB; Saboruad Dextrose supplemented with streptomycin (30°C), 96 h for yeasts; and MacConkey agar for total coliform count 37°C, 48 h for total viable counts. Incubation for LAB was done under anaerobic conditions.

### 3. RESULTS AND DISCUSSION

The primary products of fermentation are generally alcohol and organic acids such as lactic acid, acetic and propanoic acid. Table 1 shows the microbial load during and after the fermentation of maize, millet and guinea corn as well as that of their composite-based paps. Guinea corn based pap had the highest lactic acid bacteria count of  $9 \times 10^3$  CFU/g at 0 hr of fermentation which decreased to  $6.6 \times 10^5$  CFU/g at the 72<sup>nd</sup> hr. This could be due to the inhibitory effect of organic acids produced during fermentation on lactic acid bacteria. On the other hand, the composite of the three cereals had the lactic acid bacteria count of  $4.2 \times 10^4$  CFU/g at 0 hr of fermentation which increased to  $8.6 \times 10^5$  CFU/g at the 72<sup>nd</sup> hr, the highest value recorded for all samples studied. The increase in lactic acid bacteria may be attributed to tolerance to organic acid due reduced production and consequently concentration of organic acids in the product. Maize based pap had the highest total coliform count  $1.5 \times 10^1$  CFU/g at 0 hr which increased to  $2.3 \times 10^1$  CFU/g and  $4.0 \times 10^1$  CFU/g at the 24<sup>th</sup> and 48<sup>th</sup> hrs respectively. However, at the 72<sup>nd</sup> hr, the value decreased to  $3.7 \times 10^1$  CFU/g. At 0 hr, guinea corn based pap, presented a total coliform count of  $1.4 \times 10^1$  CFU/g but increased to  $2.2 \times 10^1$  CFU/g and

$4.4 \times 10^1$  CFU/g at the 24<sup>th</sup> and 48<sup>th</sup> hrs respectively. But at the 72<sup>nd</sup> hr, there was a decrease to  $4.0 \times 10^1$  CFU/g. Millet based pap sample at 0 hr had a total coliform count of  $1.3 \times 10^1$  CFU/g which increased to  $2.4 \times 10^1$  CFU/g and  $5.5 \times 10^1$  CFU/g at the 24<sup>th</sup> and 48<sup>th</sup> hrs respectively. But, at the 72<sup>nd</sup> hr, there was a decline in the total coliform count to  $4.8 \times 10^1$  CFU/g. The composite had a total coliform count of  $0.48 \times 10^2$  CFU/g at 0h which increased to  $1.0 \times 10^2$  CFU/g and  $1.9 \times 10^2$  CFU/g at the 24<sup>th</sup> and 48<sup>th</sup> hrs respectively but however decreased at the 72<sup>nd</sup> hr to  $1.5 \times 10^2$  CFU/g. These observations may be attributed to the inactivation of microbial activity by organic acids produced in the process of fermentation. This result is consistent with the finding of Hui et al. [13], which showed that butyric acid and propionic acid significantly inhibited the growth of thermophilic bacteria. Millet based pap had the highest fungi count of  $2.3 \times 10^1$  CFU/g at 0hr of fermentation which increased progressively to record the highest count of  $4.9 \times 10^1$  at the 72<sup>nd</sup> hr. However, pap derived from the composite of the cereals presented the lowest fungi count of  $0.52 \times 10^2$  CFU/g at 0hr which however increased progressively to record the highest count of  $1.4 \times 10^1$  CFU/g at the 72<sup>nd</sup> hr. This observation is in tandem with the work of Cherrington et al. [14], which affirms that the tolerance of microorganisms to organic acids depend on factors among which is microbial specie to which they belong. Seah et al. [14] affirmed that the ability of LAB strains to tolerate acid is commonly used as one of the preliminary selection criteria for potential probiotic candidates. From the outcome of this research, it is evident that the composite of the three cereals is best for pap production owing to the richness of its pap in lactic acid bacteria (probiotics) which are fundamentally known for their crucial roles in enhancing gastrointestinal health.

**Table 1. Microbial load during and after fermentation of maize, millet and sorghum for Ogi production**

Medium	Cereals	Fermentation period (hr)			
		0	24	48	72
Lactic acid bacteria count (CFU/g)	Maize	$0.7 \times 10^4$	$1.6 \times 10^5$	$6.7 \times 10^6$	$5.8 \times 10^5$
	Millet	$0.8 \times 10^4$	$1.8 \times 10^5$	$7.0 \times 10^6$	$6.4 \times 10^5$
	Guinea corn	$9.0 \times 10^3$	$1.8 \times 10^5$	$7.3 \times 10^6$	$6.6 \times 10^5$
	composite	$4.2 \times 10^4$	$5.1 \times 10^5$	$1.1 \times 10^6$	$8.6 \times 10^5$
Total coliform count CFU/g)	Maize	$1.5 \times 10^1$	$2.3 \times 10^1$	$4.0 \times 10^1$	$3.7 \times 10^1$
	Millet	$1.3 \times 10^1$	$2.4 \times 10^1$	$5.5 \times 10^1$	$4.8 \times 10^1$
	Guinea corn	$1.4 \times 10^1$	$2.2 \times 10^1$	$4.2 \times 10^1$	$4.0 \times 10^1$
	composite	$0.48 \times 10^2$	$1.0 \times 10^2$	$1.9 \times 10^2$	$1.5 \times 10^2$

Medium	Cereals	Fermentation period (hr)			
		0	24	48	72
Fungi count CFU/g)	Maize	1.2×10 <sup>1</sup>	1.9×10 <sup>1</sup>	2.8×10 <sup>1</sup>	3.3×10 <sup>1</sup>
	Millet	2.3×10 <sup>1</sup>	3.1×10 <sup>1</sup>	4.0×10 <sup>1</sup>	4.9×10 <sup>1</sup>
	Guinea corn	1.3×10 <sup>1</sup>	2.1×10 <sup>1</sup>	3.0×10 <sup>1</sup>	3.8×10 <sup>1</sup>
	composite	0.52×10 <sup>2</sup>	0.90×10 <sup>2</sup>	1.0×10 <sup>2</sup>	1.4×10 <sup>2</sup>

Table 2. pH of fermented cereals (pap) made from different cereals

Cereals	Fermentation period (hr)			
	0	24	48	72
Maize	5.60±0.3 <sup>a</sup>	5.10±0.1 <sup>a</sup>	4.70±1.2 <sup>b</sup>	4.30±0.4 <sup>b</sup>
Millet	5.50±0.8 <sup>a</sup>	5.05±0.6 <sup>a</sup>	4.85±2.3 <sup>b</sup>	4.26±0.3 <sup>b</sup>
Guinea corn	5.20±0.5 <sup>a</sup>	4.98±0.9 <sup>a</sup>	4.50±0.9 <sup>a</sup>	4.20±0.1 <sup>a</sup>
Composite	5.30±0.2 <sup>a</sup>	5.25±0.1 <sup>a</sup>	5.00±0.10 <sup>a</sup>	4.96±0.11 <sup>b</sup>

#### 4. CONCLUSION

In conclusion, this work reveals that pap production with a blend of maize, millet and guinea corn is ideal and hence should be encouraged among consumers and caregivers for its richness in probiotics (lactic acid bacteria) which are known for their potential to enhance gastrointestinal health.

#### DISCLAIMER

The products used for this research are commonly and predominantly used products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company but rather it was funded by personal efforts of the authors.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

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