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Microbial Profile of Fermented Maize Flour (Ogi) and African Oil Bean (*Pentaclethra macrophylla*) Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

This study assessed the microbial and physicochemical properties of fermented maize flour (*Ogi*) and *Pentaclethra macrophylla* seeds (*Ugba)*. The microbial analyses were done based on microbiological standards. The samples were monitored at points of preparation from 0 to 96 hours of fermentation. The physicochemical parameters were performed in accordance to standard procedures. Selected dilutions were inoculated by pour-plate method on appropriate media for isolation of aerobic bacteria, Staphylococci, coliforms, lactic acid bacteria (LAB) and fungi. Molecular identification of the LAB were done using 16s rRNA Sanger sequencing method. The pH decreased steadily from 3.9 to 3.3 for *Ogi* and from 7.7 to 5.8 for *Ugba*, with a corresponding increase in titratable acidity from 1.3 to 2.5 for *Ogi* and from 0.6 ± 0.14^{ef} to 1.2 ± 0.28^{c} for *Ugba*. Microorganisms isolated from fermented maize flour (*Ogi*) and '*Ugba*' were; *Lactobacillus brevis, L. plantarum, Pediococcus acidilactici, Staphylococcus* species*, Escherichia coli, Bacillus* species*, Micrococus* species, *Pseudomonas* species*, Proteus* species, *Saccharomyces* species*, Aspergillus* species *and Candida* species. The total bacteria counts showed decrease in both samples, with *Ugba* having the highest bacteria count ($2.7\times10^{6}\pm12.00^{a}$ cfu/g which decreased to $1.0\times10^{6} \pm 2.00^{f}$ cfu/g) compared to *Ogi* with values ($1.3\times10^{6} \pm 3.00^{c}$ cfu/g to $1.6 \times10^{5}\pm 1.73^{h}$ cfu/g) from 0 hour to 96 hours of the fermentation period. Fungi counts increased in both samples throughout the fermentation period with *Ugba* having counts of ($1.4 \times10^{3}\pm 2.65^{i}$ to $2.8 \times10^{4} \pm 5.00^{a}$ cfu/g), and *Ogi* ($7.7 \times10^{3}\pm 1.53^{h}$ to $2.7 \times10^{4}\pm 6.00^{b}$ cfu/g). LAB were persistent and most predominant in *Ogi* while *Bacillus* species were most predominant in *Ugba*. The variations in their different levels of values were of significant

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difference ($P \leq 0.05$). This study revealed the distribution of fermentative microorganisms and few contaminants which were not directly associated with fermentation process and that can however, be further reduced through heat preparation treatment of *Ogi*.

Keywords: Fermentation; microbial; microorganisms; Ogi; Ugba.

1. INTRODUCTION

Fermentation is one of the oldest and most economical methods of producing and preserving foods particularly in tropical countries whose environmental conditions favour food spoilage [1]. Fermentation of food typically involves the application of microorganisms that produces certain enzymes which changes the chemical attributes of the food from its original form [1]. In developed countries, most fermented foods are produced under controlled conditions while in developing countries such as Nigeria; such foods are processed under uncontrolled conditions, using village art methods and age-old techniques [2]. The changes that occur during fermentation could either be deleterious or beneficial [2].

Roots, legumes, cereals, fruits, oil seeds, nuts, meat, fish, milk and palm tree sap are some of the substrates from which fermented foods are derived. A variety of food products such as snacks, porridge for children and meals for adult are usually prepared from maize (cereals). One of the popular indigenous cereal-based fermented foods in Nigeria is *Ogi*. *Ogi* is a traditional porridge produced from maize, sorghum or millet grains majorly used as powerful weaning diet for children as well as dietary staple for adults in West Africa [3,4]. *Ogi* has a distinct aroma, sour taste and fine texture. Its colour depends on the type of raw materials used for the processing and can either be consumed as porridge (pap) or as a gel-like product (*agidi*) [5]. In Nigeria, '*ogi*' are called various names based on the locality and the type of cereal it is gotten from. In the Western states of Nigeria where it is obtained mostly from white maize, it is called *Ogi*. When processed from sorghum is called 'ogi-baba' and light brown in colour while from millet it is called 'ogi-gero' having a greenish to grey colour and in the Northern part of Nigeria, it is known as 'akamu' or 'eko gbona' [6,5].

Cereal products such as maize is rich in carbohydrates and minerals such as potassium and magnesium but contains trace amounts of vitamins (B-vitamins) and amino acids mainly lysine and tryptophan, and the presence of anti-nutritional factors contributes to the low content of protein [7]. During the production of ogi, a lot of nutrients such as protein, vitamins and minerals are lost, thereby adversely affecting its nutritional quality [8,9].

Microorganisms such as Lactic acid bacteria (*Lactobacillus plantarum* and *Streptococcus lactis*) and yeasts (*Saccharomyces cerevisiae,Candida* spp.) have been found to be predominant in the fermentation of ogi, where they play vital roles like, the development of aroma, microbial stability and enhancement of flavor of the fermented products [10,11]. Also, Lactic acid fermentation plays a role in reducing antinutritional factors, increasing nutrient density and antimicrobial activities in the fermented product [12].

Ugba is a protein based fermented food condiment obtained from the seeds of the African oil bean (*Pentaclethra macrophylla*) and used to complement the nutritional content of foods. *Ugba* is a product of natural fermentation involving different groups of microorganisms in the fermentation process such as *Bacillus, Micrococcus,* and lactic acid bacteria (LAB) [13,14]. It is important in diets as it provide good sources of proteins micronutrients, edible oils and fats, having high nutritive and calorific values in the foods [15]. The aim of this research is to evaluate the microbiological and physicochemical qualities of *Ogi* and *Ugba*.

2. MATERIALS AND METHODS

2.1. Sample collection

Maize grains (*Zea mays*) and African oil bean seeds (*Pentaclethra macrophylla)* used in this study were obtained from a local market in Umuahia, Abia atate, Nigeria. They were transported to the laboratory in cleaned polyethylene bags for further use and duly monitored at the points of preparation, over a period of four days, from zero (0) to 96 hours of fermentation.

2.1.1 Sample preparation and fermentation of maize seeds and African oil bean seeds

The maize grains were sorted for stones, rot and other defects. Grains without defects were

selected, cleaned and soaked in water, steeped for 24 h at room temperature (28 ± 2°C) and germinated for 48 hours. The maize grains were then, milled and sieved through a fine mesh sieve to obtain the maize flour. The samples were allowed to ferment spontaneously at ambient temperature (24 – 30°C) [16].

The hard brown testa of the oil bean seeds were soften using autoclave at 121°C temperature and 15 pounds per square inch (psi) pressure for an hour. The seeds were dehulled and the cotyledons washed, drained and rewashed. The washed cotyledons were cut into long thin slices, mixed with salt, grinded and allowed to ferment at room temperature for five days [17].

2.2 Physico-chemical Analysis

The samples were taken during fermentation and analyzed for total titratable acidity and pH at 0, 48 and 96 hours, in triplicates [18,19].

2.3 Microbiological Analysis

2.3.1 Enumeration of microorganisms in the fermented samples

This was done by the serial dilution technique where 1.0 g each of the sample was homogenized in 9.0 ml of sterile peptone water. Colony-forming units (cfu) were determined using the pour plate method. From appropriate ten-fold dilutions, 1 ml aliquots of each selected dilutions was plated in triplicate into the appropriate media for enumeration and isolation of bacteria. Nutrient agar (NA) was used for total bacteria count, MacConkey agar (MCA) for total coliform counts, Mannitol salt agar (MSA) for total staphylococcus count, De-Mann Rogosa-sharpe (MRS) agar for total lactic acid bacteria count and potato dextrose agar (PDA) for total fungi count . NA, MCA, and MSA plates were incubated at 37^0C for 24 hours; MRS plates were incubated anaerobically in an anaerobic jar at 30^0C for 48 to 72 hours while PDA plates were incubated at 28^0C for 48 to 72 hours. The colonies were counted and the data transformed into logarithms of the numbers of colony forming unit (cfu) per gram [20,21].

2.3.2 Characterization and identification of microorganisms

Colonies were randomly picked from plates used for viable counts and purified by repeated sub-culturing onto fresh sterile agar plates by streaking to obtain pure cultures. Pure isolates were stored in agar slants at 4°C for further investigation. The colonies were examined macroscopically and microscopically. Their characteristic features such as; the extent of growth, colony color and elevation, consistency, size, margin and the presence of pigments were recorded. The bacterial isolates were further subjected to standard microbiological and biochemical tests. The colony, cell morphology and standard biochemical tests of each pure culture were determined according to bacterial taxonomical methods of [22,23].

Colonies of fungal pure isolates on potato dextrose agar plates were examined and their characteristic features including the extent of growth, colour, visible mycelia, spores and pigmentation were recorded accordingly. Their structural characteristic features were observed in slide mounts of the isolates stained with lacto phenol cotton blue solution and examined under the microscope. Features such as; the natural form of outgrowth (sporangiophores and/or conidiophores), the direction of mycelia growth, presence of branching and septate were observed. The characteristics were used for identification [22].

2.3.3 Identification and molecular characterization of probiotic lactic acid bacteria by 16S rRNA gene sequencing

Presumptive LAB isolates on MRS agar were examined for their Gram staining, spore forming, motility, catalase, potassium hydroxide (KOH), oxidase status and their cell morphology [24].

The 16S rRNA genes of the probiotic LAB strains were amplified by polymerase chain reaction using a pair of 16S rRNA universal primers designated as 27F (5'- AGA GTT TGA TCC TGG CTC AG-3') for forward and 1492R (ACG GCT ACC TTG TTA CGA CTT-3') for reverse [25]. Polymerase chain reaction was performed in a 20µl reaction volume containing 2.0µl of template DNA (1µg), 10.0µl of 2× PCR master mix which contains Taq DNA polymerase, dNTPs, reaction buffer, $MgCl_2$, KCl and PCR enhancer/stabilizer; 1.0µl of forward primer (2.5µM), 1.0µl of reverse primer (2.5µM) and 6.0µl of nuclease-free water. PCR reactions were carried out in a TC-412 thermocycler utilizing amplification conditions such as: Initial denaturation step of 94^oC for 2 minutes, followed by 35 amplification cycles each consisting of denaturation at 94^oC for 30 seconds, annealing at 50.2^oC for 30 seconds and extension at 72^oC for 2 minutes. Reactions were

terminated at final extension of 72°C for 10 minutes. The amplified polymerase chain reaction (PCR) products were analysed in a 1.0% (w/v) agarose gel electrophoresis in 1x TAE buffer at 100V for 1 hour. A 1kb DNA ladder (O'GeneRuler) was used as molecular size marker. The gel was then stained with ethidium bromide solution, visualised in a gel documentation system, photographed and the amplicons purified and sequenced [26]. Sequence assembly and alignment were carried out using CLC bio software. Gene sequences were compared with GenBank database at National Centre for Biotechnology Information (NCBI) using BLASTn search tool to identify the strains.

2.4 Statistical Analysis

Triplicate determinations were carried out and standard errors were calculated for all results. One-way analysis of variance (one-way ANOVA) by using the statistical package for social sciences (SPSS Inc, Chicago, USA) program version 17.0 was used to analyze all data collected. The significant difference between the variables at $p < 0.05$ was determined by Duncan test.

3. RESULTS AND DISCUSSION

The changes in the pH of the fermenting *Ogi*, and *Ugba* samples during the 96 hours fermentation period are presented in Fig. 1. A decrease in the pH value was observed for both samples. *Ogi* decreased from 3.9 ± 0.10^{d} at 0 hr, to 3.3 ± 0.10^{f} at 96 hrs while *Ugba* values decreased from 7.7 ± 0.10^{a} at 0 hr to $5.8^{c} \pm 0.10$ at 96 hrs.

Fig. 2 shows an increase in the Titratable acidity (TTA) of the fermenting samples. *Ogi* increased from 1.3 ± 0.14^{c} at 0 hr to 2.5 ± 0.00^{a} at 96 hrs while *Ugba* increased from 0.6 ± 0.14^{ef} at 0 hr to 1.2 ± 0.28^{c} at 96 hrs.

The microbial counts of *Ogi and Ugba* samples are presented in Table 1. The total bacteria counts of both samples shows decrease in both samples, with *Ugba* having the highest bacteria count ($2.7\times10^{6}\pm12.00^{a}$ cfu/g which decreased to $1.0\times10^{6} \pm 2.00^{e}$ cfu/g) compared to *Ogi* with values ($1.3\times10^{6} \pm 3.00^{b}$ cfu/g to $1.6 \times10^{5}\pm 1.73^{f}$ cfu/g) from the start to the end of the fermentation period. The variations in their different levels of values were of significant difference. The total coliforms counts also decreased in both samples. In *Ogi,* it decreased (from $6.0\times10^{5}\pm 3.46^{c}$ cfu/g to $1.0\times10^{5}\pm 2.00^{e}$ cfu/g), and in *Ugba* (from $1.6\times10^{6}\pm5.29^{a}$ cfu/g to $6.3\times10^{5}\pm 4.00^{c}$ cfu/g). The variations in their different levels of values were of significant difference. The total LAB counts increased throughout the fermentation period in *Ogi* ($5.0 \times10^{5}\pm 4.00^{c}$ cfu/g to $1.0\times10^{6}\pm 2.6^{a}$ cfu/g) but were totally absent in *Ugba*. The variations in their different levels of values were of significant difference. The *Staphylococcus* species were eliminated after 24 hours in *Ogi* while it decreased from $8.6\times10^{5}\pm 4.00^{a}$ cfu/g to $5.1\times10^{4}\pm 2.65^{d}$ cfu/g in *Ugba*. Fungi counts increased in both samples throughout the fermentation period with *Ugba* having the highest counts of $2.8 \times10^{4} \pm 5.00^{a}$ cfu/g, and *Ogi* having counts of $2.7 \times10^{4}\pm 6.00^{b}$ cfu/g at the end of the fermentation period. The variations in their different levels of values were of significant difference ($P \leq 0.05$).

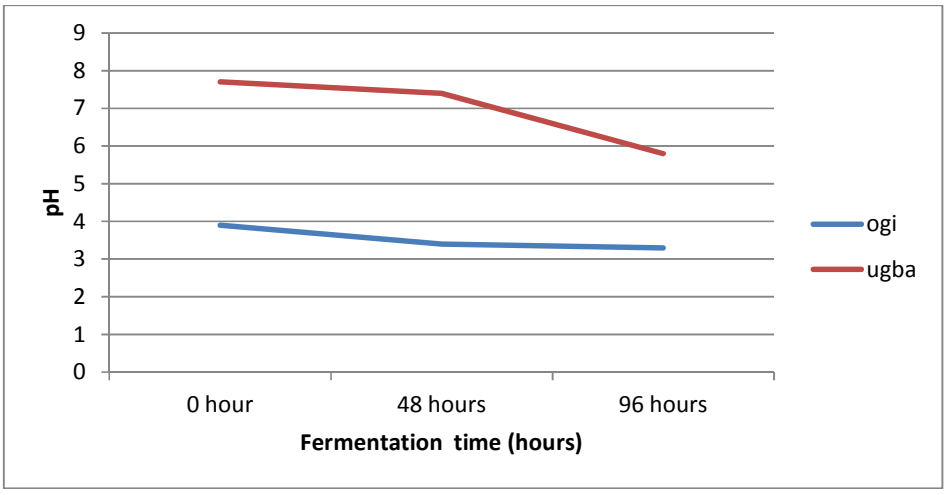

Fig. 1. pH of ogi and ugba during fermentation

Table 1. Microbial counts of *Ogi* and *Ugba* during fermentation

SAMPLES / FERMENTATION HOURS	TOTAL BACTERIA COUNTS (cfu/g)	TOTAL COLIFORMS COUNTS (cfu/g)	TOTAL LAB COUNTS (cfu/g)	TOTAL STAPHYLOCOCCUS COUNTS (cfu/g)	TOTAL FUNGI COUNTS (cfu/g)
A : 0 HOUR	$1.3\times10^{6} \pm 3.00^{b}$	$6.0\times10^{5}\pm3.46^{c}$	$5.0\times10^{5}\pm 4.00^{c}$	$2.8\times10^{5} \pm 3.00^{c}$	$7.7\times10^{3}\pm 1.53^{e}$
48 HOURS	$1.1\times10^{6} \pm 1.73^{d}$	$2.5\times10^{5}\pm 2.65^{d}$	$7.4\times10^{5} \pm 3.00^{b}$	$0.0\times10^{4} \pm 0.00^{e}$	$2.6\times10^{4} \pm 3.46^{c}$
96 HOURS	$1.6\times10^{5}\pm 1.73^{f}$	$1.0\times10^{5}\pm2.00^{e}$	$1.0\times10^{6}\pm 2.65^{a}$	$0.0\times10^{4}\pm 0.00^{e}$	$2.7\times10^{4}\pm 6.00^{b}$
B : 0 HOUR	$2.7\times10^{6}\pm12.00^{a}$	$1.6\times10^{6}\pm5.29^{a}$	$0.0\times10^{4}\pm 0.00^{d}$	$8.6\times10^{5}\pm 4.00^{a}$	$1.4\times10^{3}\pm 2.65^{f}$
48 HOURS	$1.2\times10^{6} \pm 2.65^{c}$	$1.0\times10^{6}\pm2.65^{b}$	$0.0\times10^{4}\pm 0.00^{d}$	$7.8\times10^{5}\pm 3.46^{b}$	$2.3\times10^{4}\pm 1.73^{d}$
96 HOURS	$1.0\times10^{6} \pm 2.00^{e}$	$6.3\times10^{5}\pm 4.00^{c}$	$0.0\times10^{4} \pm 0.00^{d}$	$5.1\times10^{4}\pm 2.65^{d}$	$2.8\times10^{4} \pm 5.00^{a}$

Values and means of triplicate analysis ± standard deviation. Means with different superscripts in the same column are significantly different ($P \leq 0.05$).
***Key: A** = Ogi; **B** = Ugba;*

Table 2. Biochemical characterization of Bacteria

														Sugar Fermentation							
Isolate code	**Colony features**	**Gram stain reaction**	**Cellular morphology**	**Catalase**	**Oxidase**	**Coagulase**	**Motility**	**Indole**	**Methyl red**	**Voges proskauer**	**Citrate**	**No_3**	**Spore**	**Glucose**	**Lactose**	**Sucrose**	**Maltose**	**Fructose**	**Mannitol**	**Probable organism**
1	Yellow colonies, convex smooth and entire on mannitol salt Agar	+	Clustered oval (cocci)cells	+	-	+	-	-	+	-	-	+	-	+	+	+	+	+	+	*Staphylococcus* species

2	Slightly elevated pinkish colonies on macConkey Agar but shiny black (green metallic sheen) on EMB Agar	-	Short rods	+	-	-	+	+	+	-	-	+	-	+	+	+	+	+	-	+	*Escherichia coli*
3	Partially submerged whitish colonies, rough and irregular surface on nutrient agar	+	Single rods	+	-	-	+	-	-	+	+	+	+	+	-	+	+	+	+	+	*Bacillus subtilis*
4	Flat large creamy white colonies with entire edge	+	Clustered rods	+	-	-	+	-	-	+	+	+	+	+	+	+	+	+	+	+	*Bacillus licheniformis*
5	Tiny round yellow colonies with slight elevation and entire edge	+	Oval cells in single and pairs (clus ters)	+	+	-	-	-	-	+	+	-	-	-	-	-	-	-	-	-	*Micrococus* species
6	Creamy, slimy colonies with bluish-green pigments on NA	-	Short rods	+	+	-	+	-	+	-	+	+	-	+	-	-	-	-	-	+	*Pseudomonas* species
7	Creamy, moist, smooth round colonies with entire margin on MRS Agar	+	Long rods in chain	-	-	-	-	-	-	+	-	-	-	+	+	+	+	+	+	-	*Lactobacillus* species
8	Large swarm creamy colonies translucent on NA	-	Short rods	+	-	-	+	-	+	-	+	+	-	+	-	-	-	+	-	-	*Proteus* species

Table 3a. Morphological and biochemical characteristics of selected potential Lactic acid bacteria strains

CHARACTERISTICS	ISOLATE CODES		
	ISO 1	ISO 3	ISO 4
Gram staining	+	+	+
Colony Morphology	Creamy, moist, circular, slightly elevated, translucent colonies with slimy consistency and entire edge (margin)		
Cell shape	Long rods in chain	Long rods in chain	Coccus
Motility	–	–	–
Catalase	–	–	–
Potassium hydroxide (KOH)	+	+	+
Spore	–	–	–
Oxidase	–	–	–

Table 3b. Molecular identification of selected Lactic acid bacteria strains

Isolate codes	Similarity/E-score	Gene Bank Accession Number/Top hit	Identity Of Isolate Obtained
ISO 1	88%/0	EU626011.1	*Lactobacillus brevis*
ISO 3	74%/4E	KY817129.1	*Lactobacillus plantarum*
ISO 4	89%/0	KT725823.1	*Pediococcus acidilactici*

Table 4. Characterization of Fungi

ISOLATE	COLONY FEATURES	STRUCTURAL FEATURES	SUSPECTED FUNGI
A	Profuse growth of dark brownish colonies with visible mycelia and numerous dust-like covering and whitish margin.	Upright conidiophore that is septate, without branching and ending in a swollen head bearing phialides with many dark colored spores.	*Aspergillus* species
B	Creamy white colonies without visible mycelia but with moist surface, flat, smooth without pigment having a characteristic odour.	Round single cell, some of which possess attachment of daughter cells (buds).	*Saccharomyces* species
C	Colonies appears as large, round, creamy-white color, raised, entire, smooth and butyrous having a characteristic odour.	Spherical to sub-spherical budding yeast-like cells (blastoconidia).	*Candida* species

The bacteria identified were *Staphylococcus* species*, Escherichia coli, Bacillus* species, *Micrococcus* species*, Pseudomonas* species*, Lactobacillus* species and *Proteus* species. The fungal isolates were *Aspergillus* species*, Saccharomyces* species and *Candida* species*.*

Table 3, shows the presumptive identification of LAB isolates that were subjected to molecular characterization and identification based on 16S rRNA gene sequencing identifying them as *Lactobacillus brevis, Lactobacillus plantarum* and *Pediococcus acidilactici.*

Table 5 shows the microbial occurrence during fermentation of the samples. In *Ogi*, LAB were present in increasing numbers throughout the fermentation period, *Staphylococcus* species were eliminated after 24 hours of the fermentation period while *Micrococcus* species and *Pseudomonas* species were eliminated after 48 hours of the fermentation period. *Bacillus* species and *Escherichia coli* were present in reduced numbers at the end of the fermentation period while *Proteus* species were absent throughout the fermentation. In *Ugba*, *Bacillus* species were increasing steadily throughout the

fermentation period. *Escherichia coli, Micrococcus* and *Pseudomonas* species were moderately present while *Proteus* species were slightly present throughout the fermentation period. *Staphylococcus* species were present in reducing numbers and LAB were absent throughout the fermentation period. *Saccharomyces* species and *Candida* species were present in increasing numbers throughout the fermentation period in both samples while *Aspergillus* species were eliminated after 24hours of the fermentation period.

In this study, the pH of the samples decreased with a corresponding increase in titratable acidity as the fermentation progressed from 0 hour to 96 hours. This observation is in agreement with previous studies on *Ogi* fermentation such as those by [27,28,29]. This may be attributed to enhanced metabolic activities leading to the production of organic acids (lactic acid by fermentative organisms) in the fermenting slurries [30]. Microbial activities during fermentation lead to improved nutritional content of the fermented product.

The early rise in titratable acidity and reduced pH turns the medium acidic and prevent the proliferation of undesirable organisms that may result in poor fermentation [31,28]. Previous report has also shown that such a short fermentation period is desirable in achieving a good and consistent quality fermented product.

This study revealed high total bacterial count which decreased gradually as the fermentation progressed. This may be attributable to the availability of some nutritional components of grains and legumes that is easily metabolizable by these organisms and essential for their growth [32]. A steady increase in the lactic acid bacteria count throughout the fermentation process could be due to an increase in the acidity and the anaerobic condition of the fermenting medium as LAB usually turns medium acidic and, thus, favors the growth of only facultative anaerobes, and/or aciduric organisms. It may also be as a result of the inhibitory effect of antimicrobial products released by the lactic acid bacteria into the fermenting medium which antagonizes the growth or proliferation of spoilage microorganism [33,32]. This explains the gradual decrease in the microbial load of bacteria. The yeasts population in both samples increased steadily throughout the fermentation period which could be attributed to their ability to adapt to the condition for its growth created by the decrease in the pH (Fig. 2).

The succession of the microbial population shows a wide variation of microorganisms such as Gram negative and positive bacteria, fungi, lactic acid bacteria and yeasts associated with the fermentation of fermented maize flour (*Ogi*) and *Ugba*. The bacterial inoculum responsible for fermenting grains maybe derived from the cereal grains [16,34]. These microorganisms found in this study could originate from the cereals, legume seed composition, air, utensils, skin of the handlers, the leaves used for packaging the *Ugba* and possibly the water used for mixing.

Bacteria isolated in this study were *Staphylococcus* species, *Bacillus* species, *Escherichia coli, Micrococcus* species, *Pseudomonas* species, *Lactobacillus* species and *Proteus* species. It was observed that as pH decreased and total titratable acidity increased, the population of bacteria in *Ogi* decreased as the medium became more acidic since they are acid intolerant. Although, *Escherichia coli, Micrococcus* and *Pseudomonas* species persisted to the end of the fermentation but they were in reduced numbers. *Staphylococcus* species were eliminated after 24hours of the fermentation period. This corroborated with reports of [9] and [35]. The *Bacillus* species isolated in this study persisted to the end of the fermentation. Previous reports have also isolated *Bacillus* species in the fermentation of maize for *Ogi* production, and have been reported to show saccharolytic activities [10].

In *Ugba*, the bacteria population was higher than that observed for *Ogi*, with *Bacillus* species being the predominant bacteria. This observation is in-line with previous reports which demonstrated the predominance of *Bacillus* species in other fermenting legume proteins. *Bacillus subtilis, B. pumilus, B. megaterium* and *B. lichenformis* have also been isolated from *Ugba* fermentation, with *Bacillus subtilis* being the predominant species that plays important roles in modifying the substrate biochemically, nutritionally and sensorilly during the fermentation process [36]. *Bacillus* species isolated from fermenting leguminous sources are reported to be proteolytic and are able to breakdown oils [37]. *Escherichia coli, Micrococcus* and *Pseudomonas* species persisted to the end of the fermentation but they were in moderately reduced numbers. The population of *Staphylococcus* species decreased as the fermentation progressed but persisted to the end of the fermentation in reduced numbers while *Proteus* species was slightly present throughout the fermentation

period (Table 5), all contributing to the ecology of fermenting *Ugba*. It is evident that the production of fermented condiments is mediated by diverse microbial flora and these microorganisms utilize the nutritional components of the seeds, converting them into products that contribute to the chemical composition and taste of the condiment [14,38].

This study also confirmed the major involvement of LAB in *Ogi* fermentation which increased tremendously in counts throughout the fermentation period. They were mainly obligatory or facultative hetero fermentative and homo fermentative rods and cocci (*Lactobacilli* and *Pediococcus* spp.) In this study, species designations of LAB isolated from *Ogi* by phenotypic and genotypic characterization was confirmed by 16S rRNA gene sequencing to be predominately *Lactobacillus plantarum, Lactobacillus brevis and Pediococcus acidilactici* (Table 3). Their growth was accompanied by increase in organic acid production, decreasing the pH and resulting in acidification of the product and making the environment ideal for their growth, thereby, predominating and preventing the growth of other undesirable microorganisms. This is in-line with other studies on fermented products which report LAB as the predominant microorganisms in the fermentation of products such as kenkey, Garri, Agbelima, identifying *L. plantarum* mostly [33,39].

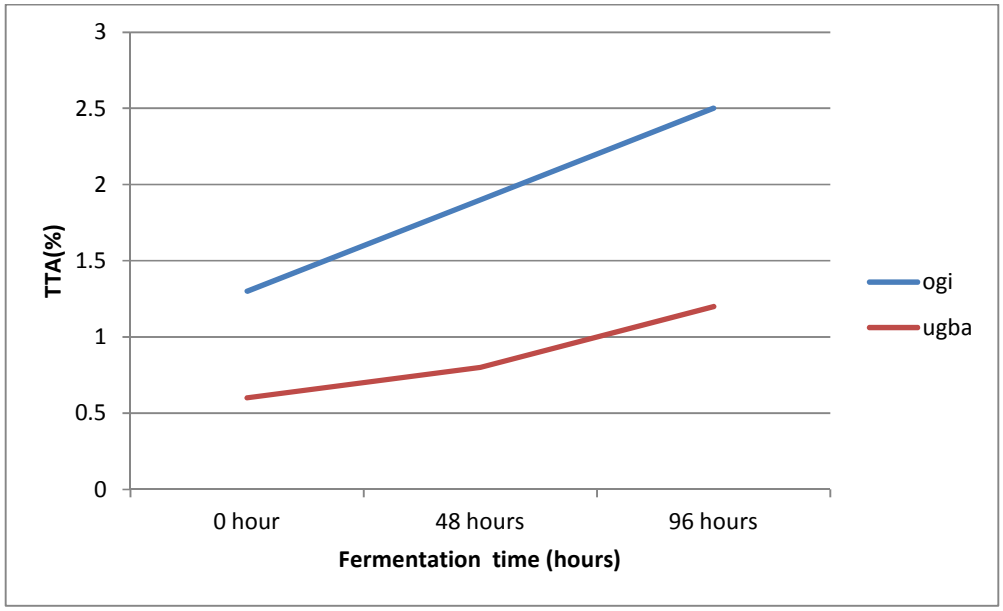

Fig. 2. Titratable acidity (TTA) of ogi and ugba during fermentation

Table 5. Microbial occurrence during fermentation of *Ogi* and *Ugba*

ISOLATES	OGI			UGBA		
HOURS	**0**	**48**	**96**	**0**	**48**	**96**
Staphylococcus species	+	_	_	+++	++	+
Bacillus species	++	+	+	++	+++	+++
Escherichia coli	+	+	+	++	++	++
Micrococcus species	+	+	_	++	++	++
Pseudomonas species	+	+	_	+	++	++
Lactobacillus species	+	++	+++	_	_	_
Proteus species	_	_	_	+	+	+
Saccharomyces species	+	++	+++	+	++	+++
Candida species	+	++	+++	+	++	+++
Aspergillus species	+	_	_	+	_	_

Key: + = Present slightly; ++ = Present moderately; +++ = Present much; - = Absent

The undesirable microorganisms such as coliforms and molds (*Aspergillus* spp.) which were present at the start of fermentation were gradually decreasing and some totally eliminated (Table 5). This observation agrees with previous research findings by [40] and [41] that molds which are present at the initial stage of *Ogi* fermentation are subsequently eliminated. The subsequent disappearance of molds after 24 hours in this study may be due to the low oxygen condition in the fermenting material as a result of the production of organic acids by lactic acid bacteria [42]. The mold *(Aspergillus* spp.) isolated appear insignificant in the fermentation process as it does not appear to play any important role in it, rather occurring as a common contaminants, which can only have effect on the flavor of the final products when they occur in high numbers.

The yeasts population in both samples, increased steadily throughout the fermentation period which could be attributed to their ability to adapt to the condition for its growth created by the decrease in the pH. This is in accordance with the findings previously reported for other fermented beverages [43]. *Saccharomyces* species and *Candida* species were found in this study which reiterates the findings in other studies that have reported several yeasts species being found in spontaneous lactic fermenting cereals such as species of *Saccharomyces* and *Candida* [40]. *Saccharomyces* spp. was the dominant species associated with this fermentation as this is considered the most reported in African indigenous fermented foods and beverages [44]. Yeasts have also been reported by several authors [40,41] in African traditional fermented products to co-exist and carry out symbiotic association with lactic acid bacteria. They play a role in enhancing typical flavor and aroma of these fermented products as well as show enzymatic ability to break down maize starch and allowing better access to their nutritional content resulting in stability of the final products [41].

4. CONCLUSION

This study revealed the distribution of fermentative microorganisms and some pathogens /contaminants (microbial loads) which were not directly associated with fermentation process but can however, be further reduced through heat treatment as *Ogi* is usually boiled or treated with boiled water before consumption.

DISCLAIMER

The products used for this research are commonly and predominantly use 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 rather it was funded by personal efforts of the authors

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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