PRINT ISSN 1119-8362
Electronic ISSN 2659-1499

Full-text Available Online at
https://www.ajol.info/index.php/jasem
https://www.bioline.org.br/ja

J. Appl. Sci. Environ. Manage.
Vol. 28 (12) 4095-4103 Dec. 2024

Microbial And Proximate Properties Of Lime Juice Bio-Preserved Ogi Procured From New Benin Market, Benin City, Edo State, Nigeria

***[1]NGWOKE, CA; [2]IGIEBOR, FA**

**[1]Department of Microbiology, Faculty of Science, Benson Idahosa University, Benin City, Nigeria*
[2]Department of Microbiology, Faculty of Science, Delta State University, Abraka, Nigeria

**Corresponding Author email: cngwoke@biu.edu.ng*
**ORCID: https://orcid.org/0009-0000-3312-5879*
**Tel: +2348033768246*

Co-Author Email: francis.igiebor@lifesci.uniben.edu

ABSTRACT: Ogi, made from maize cereal is widely used in West Africa, especially as a weaning diet for children and could be preserved inwith the water decanted after every 24hours or in the freezer. However, these preservative methods are labour intensive or expensive. Hence, the objective of this paper is to evaluate the microbial and proximate properties of lime juice bio-preserved Ogi procured from New Benin Market, Benin City, Edo State, Nigeria using appropriate standard techniques and procedures. Bacteria isolates from both the control and test samples were characterized by cultural methods in appropriate media and identified based on their morphological and biochemical characteristics. Proximate analysis of the samples stored with lime juice and that of the control, stored with water was also carried out. The total bacteria count for samples stored at room temperature (28±2°C) ranged from 2 x 10^3 to 33.3 x 10^3CFU/g, while the fungal count was between 12 x 10^3 to 29 x 10^3 CFU/g. Organisms identified from the control sample were *Lactobacillus* sp, *Lueconostocs* sp and *Staphylococcus aureus*. The experimental samples yielded *Corynebacterium* sp, *Flavobacterium* sp and *Klebsiella* sp. The presence of lime inhibited the growth of some organisms but did not affect the variety of microorganisms present. Determination of the proximate analysis of the samples stored with lime juice and that of the control, stored with water showed no significant difference in the composition of the nutrients. However, the samples treated with lime juice had a longer shelf life and better organoleptics.

DOI: https://dx.doi.org/10.4314/jasem.v28i12.20

License: CC-BY-4.0

Open Access Policy: All articles published by **JASEM** are open-access articles and are free for anyone to download, copy, redistribute, repost, translate and read.

Copyright Policy: © 2024. Authors retain the copyright and grant **JASEM** the right of first publication. Any part of the article may be reused without permission, provided that the original article is cited.

Cite this Article as: NGWOKE, C. A; IGIEBOR, F. A. (2024). Microbial And Proximate Properties Of Lime Juice Bio-Preserved Ogi Procured From New Benin Market, Benin City, Edo State, Nigeria. *J. Appl. Sci. Environ. Manage.* 28 (12) 4095-4103

Dates: Received: 22 October 2024; Revised: 20 November 2024; Accepted: 08 December 2024; Published: 18 December 2024

Keywords: Ogi (*Zea maize* flour); Lime juice; Lime fruit; Organoleptics; Biopreservation.

Food preservation is fundamental for enhancing food security and reducing spoilage, particularly in regions with limited access to refrigeration or advanced preservation technologies. Microbial activity remains a leading cause of food spoilage, manifesting in altered sensory attributes such as appearance, flavour, and odour, which render food unfit for consumption. The rapid spoilage of perishable foods like ogi, a fermented cereal product made primarily from maize (*Zea mays*), poses significant challenges, especially in low-resource settings. Traditional preservation methods, such as refrigeration and chemical additives, are effective but often inaccessible or undesirable due to cost and health concerns (Ibrahim *et al*., 2020; Eze *et al*., 2022).Lime (*Citrus aurantiifolia*) has gained attention as a natural biopreservative owing to its antimicrobial properties, which are attributed to bioactive compounds like citric acid, flavonoids, and essential oils. These compounds disrupt microbial cell walls and enzyme

**Corresponding Author email: cngwoke@biu.edu.ng*
**ORCID: https://orcid.org/0009-0000-3312-5879*
**Tel: +2348033768246*

systems, effectively extending the shelf life of perishable products (Jittanit *et al*., 2020). Lime has been successfully used in preserving various food products, including fruit juices, where it has demonstrated efficacy in reducing microbial loads and improving sensory attributes (Adetunji *et al*., 2022). Despite this, its potential for preserving traditional foods like ogi has not been extensively studied. Ogi is a staple food in West Africa, commonly used as a weaning diet for infants. Its traditional preservation method involving immersion in water and daily decantation is insufficient, leading to spoilage within days. This highlights the need for alternative, cost-effective preservation techniques. Incorporating lime juice into ogi preservation could offer a natural, affordable solution for extending shelf life, particularly in rural areas with limited refrigeration (Okeke *et al*., 2021). Hence, the objective of this paper is to evaluate the microbial and proximate properties of lime juice bio-preserved Ogi procured from New Benin Market, Benin City, Edo State, Nigeria.

MATERIALS AND METHODS

Collection of Samples: Ogi samples were procured from New Benin Market, Benin City, Edo State, Nigeria, and transported to the laboratory under aseptic conditions. Fresh, green limes (*Citrus aurantiifolia*) were also purchased from the same market. In the laboratory, the limes were cleaned with 99% ethanol before juicing..

Five sterile conical flasks were each filled with 100 g of ogi. A control sample (Sample A) was preserved with 1 L of distilled water, while experimental samples (B, C, D, and E) were treated with 120 mL, 140 mL, 160 mL, and 180 mL of freshly prepared lime juice, respectively. All flasks were sealed with cotton wool wrapped in aluminium foil and stored at room temperature (28 ± 2°C).

Microbiological Analysis: The pour plate method was used to determine bacterial counts. A three-fold serial dilution was prepared using sterile distilled water, and 1 mL of the 10^{-3} dilution was plated onto nutrient agar. Plates were incubated at 37°C for 24 hours. Colonies were counted and recorded following standard procedures (Igiebor and Uwuigiaren, 2024).

Isolation of Bacteria: Three-fold serial dilution method was used to get countable colonies. Five test tubes containing 9ml of distilled water were prepared. 1ml of sample B was transferred into the first appropriately labelled test tube, from which 1ml was pipette into the second test tube and then into the third test tube and also from the third test tube to the fourth test tubes, So a 10^{-1}, 10^{-3}, 10^{-5}, (1:3) serial dilution was obtained from the samples.

1ml aliquot of the 10^{-3} dilution of the samples was pipette into three sets of sterile Petri dishes. 20ml of cooled nutrient agar was aseptically poured into each set of plates and gently stirred to ensure even distribution of bacteria. The agar was allowed to set and the plates were then incubated at room temperature for 24hrs, after which the colonies formed on the plates were counted. The number of colonies obtained was multiplied by the reciprocal of the dilution factor to obtain bacteria concentration of 1ml of the sample used. The result obtained was calculated using the equation 1 and expressed as colony forming units per gram (CFU/ml).

$$\mathrm{CFU/m} = \mathrm{NC} \times \frac{1}{DF} x \frac{1}{VP} \quad (1)$$

Where NC = number of colonies; DF = dilution factor; VP = volume plate

The bacteria colonies which appeared on the plates were purified by sub culturing onto fresh nutrient agar plates by the streak plate method. Distinct colonies which developed were transformed onto nutrient agar slants and stored on stock cultures for identification tests (Osarumwense and Igiebor, 2018).

Isolation of fungi: The medium used was potato dextrose agar (PDA) in which streptomycin antibiotic was added. 1ml aliquot was taken from the 10^{-3} earlier made into 3sets of Petri dishes. 12-15ml of molten potato dextrose agar was poured into the plates and allowed to solidify. 2 drops of streptomycin was incorporated into the plates to prevent bacteria growth. The plates were incubated at room temperature for 1-3days. The resulting fungal colonies were counted and later subcultured onto freshly prepared PDA plates. Distinct fungal colonies which developed were stored in a refrigerator in PDA slants for further identification procedures (Maduka *et al*., 2022).

Characterization of fungal isolates: Microscopic examination was carried out by using a drop of lactophenol cotton blue stain on a clean glass slide. With the aid of a sterile needle, a small portion of the fungus was transferred into the lactophenol cotton blue stain on the slide. A cover slip was placed over the suspension and pressed firmly but gently to exclude air bubbles. The wet mount preparation was observed under a light microscope, viewed with x10 and then x40 objectives. The shape, cell arrangement and structural characteristics were recorded. Macroscopic features like the texture of the colony,

its surface colour and the production of pigment seen on the reverse side of the Petri plate also aided in identification of fungal isolates.

Characterization of Bacteria Isolates: The cultural characteristics of various isolates were observed after appropriate incubation. The cultural characteristics used were colony size, elevation, surface appearance, colour, optical appearance and edge. The morphological characteristics of the bacteria isolates were also determined.

Biochemical Tests for the identification of Bacteria Isolates: The following biochemical tests were conducted: oxidase test, urease test, citrate utilizationtest, indole production test, catalase, coagulase, voges proskauer tests, methyl red test and sugar utilization test.

Moisture determination: Apparatus - silica dish or crucible, hot air-drying oven and desiccators.
The moisture can was first weighted empty (W_o), after which 2g of the sample material was added and both the moisture can and the sample was weighed again (W_1). This was dried in the hot air-drying oven at 105 – 110°C for 24hrs, and was cooled in a desiccator. The moisture can with the dry sample was weighed again (W_2) and returned to the oven for further 24hours to make sure that the drying was complete. It was weighed again to make sure that the weight (W_3) was constant otherwise; the drying is continued until a constant weight is obtained.

Determination of Fat: Apparatus - condenser Soxhlet extraction unit, hot air-drying oven, 250ml capacity extraction or boiling flask, desiccator, balance, dry porous thimble, water bath, shaped no 4-filter paper and cotton wool. The reagent used was petroleum ether. A 250ml extraction flask was oven dried at 105 – 110°C and weighed after cooling 0.5 – 2.0 g of the sample was weighed into a labelled porous thimble and the thimble was covered with clean white cotton wool. 200ml of petroleum ether was added into the dry 250ml extraction flask. The covered porous thimble was then placed into the condenser and the apparatus was assembled. Extraction was done for about 5-6 hours, after which the porous thimble was removed with care and the petroleum ether, collected in the top container (tube) for re-use. The extraction flask containing the oil or fat was removed from the water bath when it was almost free of petroleum ether and oven dried at 105 – 110°C for one hour, after which it was cooled in the desiccator and then weighed.

Estimation of Carbohydrate in the solid sample: Reagents: 5% phenol solution, concentrated sulphuric acid solution, 1.0 % sample solution and standard carbohydrate solution (30 µg/ml). A 0.1ml of the sample solution was pipette unto five different tubes labelled accordingly, to which 0 – 5ml of distilled water was added to give a 1ml volume of sample solution, 10ml of 5% phenol reagent solution was then added and all the tubes were put in an ice bath of 4% temperature for about 30 minutes. The samples were thoroughly mixed after which 5ml of concentrated H_2S0_4 was slowly added along the side of each test tube in the ice bath. This served as the experimental sample. A standard sample was also prepared as stated above, except that 0.1ml of the sample was replaced with 0.1ml of standard carbohydrate solution. Finally, a blank sample was prepared as stated above except that the sample was replaced with 1ml of distilled water. All the tubes for the experimental, standard and blank samples were put into a boiling water bath at 100°C and boiled for 5 – 10minutes, after which the samples are cooled down in the ice bath before reading in the spectrophotometer at 485nm.

Determination of Crude Protein: Reagents: Kjeldah tablets (selenium oxide, $NaS0_4$ + $CuSO_4$), concentrated sulphoric acid solution, Nesler's reagent (as bought), solid sample and standard $(NH_4)SO_4$ solution (200ug/ml)
A known weight of the solid sample (1gm) was transferred into a Kjeldah flask, while Kjeldah Tablets (1 – 5gm) and 20mls of concentrated sulphuric acid were added. The mixture in the flask was heated on a kjeldah digesting heater until the solution became clear. This was cooled down to room temperature before it was diluted to 50mls in a volumetric flask. The ammonium sulphate produced was estimated and used to calculate the Nitrogen in the $(NH_4)_2SO_4$ before converting the nitrogen to protein using 6.25 factor. The amount of crude protein was obtained by multiplying the nitrogen content by 6.25. This factor is based on the assumption that all feed protein contains 16% nitrogen and that all the nitrogen in the tissue is present as protein

Ash Determination: Apparatus: Crucible or silica dish, weighing balance, Muffle furnace, spatula and dessicator. The crucible was weighed empty (W_o) after which the sample was added and both the sample and crucible, weighed again (W_1). It was put in the muffle furnace at 500-600°C for 3hours and afterwards, cooled in a desiccator. The crucible and dry sample was weighed again (W_2).

Estimation of Crude fibre: Apparatus: muffle furnace, one litre conical flask, weighing balance, poplin cloth, hot air-drying oven, Buchner filtration unit, crucible or silica dish and spatula. Reagents: 125% H_2SO_4 and 1.25% NaOH. A 0.50 – 2.00g of the ground sample was weighed into a one litre conical flask and 200ml of 1 – 25% H_2SO_4 was added and boiled gently for 30 minutes. The sample was filtered through muslin cloth of poplin material, stretched over a 9cm funnel and rinsed well with hot distilled water. The material was scrapped back into the flask with a spatula and 200ml of 5% NaOH was added and allowed to boil again for 30minutes and afterwards, filtered through poplin cloth, after which it was rinsed once more with 10% HCl and twice with industrial methylated spirit, acetone or ethanol. It was finally rinsed three times with petroleum ether and allowed to drain dry. The residue was scraped into a crucible or silica dish and dried overnight at 105°C in the oven and cooled in a desiccator, after which the sample was weighed (W_1). It was ashed at 550°C for 90minutes in a muffle furnace after which it was cooled in a desiccator and weighed again (W_2).

Organoleptic test: A panel of three individuals was set up and made to sample the ogi porridge. They were to grade it according to colour, aroma, appearance, taste and viscosity. The score parameters used were: very bad, bad, good, very good and excellent. The score parameters were used to determine the level of palatability and as such, possible commercial acceptability of this method of preservation.

Determination of pH: Determination of pH values was done, using a glass electrode with combined sensing and refractive elements, inserted into the samples, connected to a pH meter. The radiometer was capable of measuring temperature dependent pH values to an accuracy of two decimal places. The radiometer was calibrated daily before and after sample measurement by reading its response to a buffer of known pH. The pH determination of the five samples was done every 24 hours.

RESULTS AND DISCUSSION

Table 1 showed the effect of lime juice on the bacterial load of ogi samples stored at room temperature (28 ± 2°C) over 24, 48, and 72 hours. Sample A (control), prepared without lime juice, consistently showed the highest bacterial count across all time intervals, starting at 33.3 x 10³ CFU/g after 24 hours and decreasing to 10.7 x 10³ CFU/g by 72 hours. Conversely, the samples treated with lime juice (B, C, D, and E) demonstrated a progressive reduction in bacterial count that correlated with the increasing concentration of lime juice. Sample B (100 g ogi/120 mL lime juice) exhibited a bacterial count of 29.3 x 10³ CFU/g at 24 hours, reducing to 9.0 x 10³ CFU/g at 72 hours. Samples C and D, with 140 mL and 160 mL of lime juice respectively, showed more pronounced bacterial inhibition, with D reducing from 23.0 x 10³ CFU/g at 24 hours to 4.3 x 10³ CFU/g at 72 hours. Sample E (100 g ogi/180 mL lime juice) demonstrated the strongest antimicrobial effect, with no detectable bacteria at 24 hours and a final count of 2.0 x 10³ CFU/g at 72 hours. This trend suggests that higher concentrations of lime juice enhance its inhibitory effect on bacterial growth.

Table 1: Total bacteria count (cfu/g) of ogi samples stored at room temperature (28±2°C)

Sample Type	24	48	72
A	33.3 x 10^3±0.9	22.3 x 10^2± 0.33	10.7 x 10^3± 0.67
B	29.3 x 10^3± 0.7	20.3 x 10^3±0.9	9.0x 10^3± 0.58
C	25.3 x 10^3± 0.34	17.7 x 10^3±0.9	7.0 x 10^3± 0.58
D	23.0 x 10^3± 0.58	15.0 x 10^3±0.58	4.3 x 10^3± 0.9
E	-	13.0 x 10^3±0.58	2.0 x 10^3± 0.58

A = 100g/1litre of water (Control); B = 100g/120ml of lime juice; C = 100g/140ml of lime juice; D = 100g/160ml of lime juice; E = 100g/180ml of lime juice

The total fungal count after 72 hours of incubation varied significantly across the samples, as indicated in Table 2.

Table 2: Total fungal count after 72hrs of incubation

Sample	CFU/ml	Mean + SE
A	30 x 10^3	29 x 10^3+0.58
	29 x 10^3	
	28 x 10^3	
B	27 x 10^3	25.7 x 10^3 +0.67
	25 x 10^3	
	25 x 10^3	
C	23 x 10^4	22.7 x 10^4 + 10.33
	23 x 10^4	
	22 x 10^4	
D	20 x 10^3	18.3 x 10^3 + 1.20
	19 x 10^3	
	16 x 10^3	
E	14 x 10^3	12 x 10^4+ 1.16
	12 x 10^3	
	10 x 10^3	

Sample A recorded the highest fungal load among the samples with a mean of 29×10^3CFU/ml±0.58, closely followed by Sample B with a mean fungal count of 25.7×10^3CFU/ml±0.67. Sample C showed a substantial increase in fungal load compared to the others, with a mean count of 22.7×10^4CFU/ml±10.33. Sample D exhibited a moderate fungal load with a mean of

18.3×10^3CFU/ml±1.20, while Sample E displayed the lowest fungal load, recording 12×10^3CFU/ml±1.16. The variations in fungal count among the samples suggest differences in their susceptibility to fungal growth under the given incubation conditions.

Table 3 showed that Lactobacillus sp was consistently present in all samples, indicating its dominance and potential role in the fermentation process. The control sample (A) contained a diverse bacterial population, including *Staphylococcus aureus*, *Lactobacillus* sp, and *Leuconostoc* sp. In the lime-treated samples (B, C, D, and E), the bacterial diversity decreased, with isolates like *Klebsiella* sp, *Corynebacterium* sp, and *Flavobacterium* sp observed alongside Lactobacillus sp, suggesting a selective inhibitory effect of lime juice on certain bacteria.

Table 3: Bacteria isolates identified

Samples	Isolates
A	*Staphylococcus aureus, Lactobacillus* sp *and Leuconostoc* sp
B	*Klebsiella*sp and *Lactobacillus* sp
C	*Corynebacterium* sp, *Lactobacillus* sp
D	*Flavobacterium* sp *Lactobacillus* sp
E	*Klebsiella* sp *Lactobacillus* sp

Table 4 demonstrates that *Aspergillus niger* and *Saccharomyces* sp were present in all samples, indicating their resilience and ability to thrive in the acidic medium of ogi. The control sample (A) exhibited the highest fungal diversity, including *Fusarium* sp in addition to *Aspergillus niger* and *Saccharomyces cerevisiae*.

However, the lime-treated samples (B, C, D, and E) showed reduced fungal diversity, with *Aspergillus niger* and *Saccharomyces* sp as the predominant isolates. This suggests that while lime juice effectively reduced bacterial diversity, its impact on fungal diversity was less pronounced, potentially due to fungi's ability to thrive in acidic conditions.

Table 4: Fungal isolates identified

Samples	Isolates
A	*Aspergillusniger, Fusarium* sp, *Saccharomyces cerevisae*
B	*Aspergillusniger, Saccharomyces cerevisae*
C	*Aspergillusniger, Saccharomyces* sp
D	*Aspergillusniger. Saccharomyces* sp
E	*Aspergillusniger, Saccharomyces* sp

The proximate composition of ogi samples stored with varying proportions of lime juice was assessed over four weeks (Table 5). The moisture content decreased progressively across all samples over the four-week storage period. The control (Pap 100/100 H_2O) consistently exhibited higher moisture levels compared to lime juice-treated samples. The control started at 66.67 % moisture in the first week and reduced to 56.63 % by the fourth week. In contrast, lime juice-treated samples, particularly those with higher lime concentrations, demonstrated significantly lower moisture content, stabilizing around 50–52 % by the fourth week. This suggests that lime juice reduces moisture retention in ogi, potentially limiting microbial activity and spoilage. The lipid content remained relatively stable for lime-treated samples (averaging 0.656 %), while the control showed a steady decline, dropping to 0.08% by the fourth week. This stability in lipid content in treated samples suggests that lime juice inhibits lipid-degrading microorganisms, likely contributing to the preservation of fat content in ogi. Carbohydrate content showed negligible variation across all samples, with minimal differences between the control and lime-treated ogi. The sample mean carbohydrate content was 97.22 %, slightly higher than the control mean of 97.05 %. This consistency indicates that carbohydrate utilization by microorganisms was limited, likely due to the antimicrobial effects of lime juice. Protein levels were more stable in lime-treated samples than in the control. While the control's protein content dropped significantly to 0.963 % by the fourth week, the treated samples maintained higher levels, averaging 1.592 %. This stability may be due to the inhibition of proteolytic organisms by the lime juice, which preserved the nitrogenous compounds in the ogi. Ash content remained consistent across all samples, with no significant changes noted between the control and treated ogi. Similarly, fibre content exhibited no noticeable differences, maintaining an average of 0.523 % for treated samples and 0.477% for the control. This consistency aligns with the observation that lime juice does not significantly affect the mineral and fibre composition of ogi.

The organoleptic test results presented in Table 6 assess various sensory attributes, colour, aroma, appearance, taste, and viscosity of five different ogi samples (A, B, C, D, E). Samples C, D, and E were rated as "Excellent," indicating a desirable and appealing colour. Sample B was rated as "Good," while sample A was not rated for colour. The aroma of samples C, D, and E was rated "Excellent," reflecting a pleasant and appealing fragrance. Sample B had a "Bad" aroma, while sample A was not rated for aroma. Samples C and D were rated "Good" in appearance, while sample E was rated "Excellent." Sample B had a "Bad" appearance, and sample A was not rated for appearance. Samples C, D, and E received "Excellent" and "Good" taste ratings,

indicating favourable taste. Sample A was rated as "Very bad," while sample B was not rated for taste. Sample E was rated "Excellent" for viscosity, while sample D was rated "Good." Sample B had a "Bad" viscosity, and sample A was not rated for this parameter.

Table 5: Proximate composition of ogi samples

	Sample Description	% Moisture	% Lipid	% Ch_2o	%Protein	%Ash	%Fibre
				1st Week			
1	Pap 100/100 H_2O	66.67	1.20	96.564	1.675	0.56	0.001
	Pap 20/80 lime Juice	60.00	0.40	97.395	1.644	0.56	0.001
	Pap 40/60 lime juice	52.38	0.60	97.164	1.675	0.56	0.001
	Pap 60/40 lime juice	52.62	0.80	96.933	1.706	0.56	0.001
	Pap 80/20 lime juice	52.63	1.00	96.764	1.675	0.56	0.001
				2nd Week			
2	Pap 100/100 H_2O	61.58	1.20	96.568	1.671	0.56	0.001
	Pap 20/80 lime juice	57.64	0.40	97.397	1.642	0.56	0.001
	Pap 40/60 lime juice	51.47	0.60	97.167	1.672	0.56	0.001
	Pap 60/40 lime juice	51.62	0.80	96.936	1.703	0.56	0.001
	Pap 80/20 lime juice	51.51	1.00	96.767	1.672	0.56	0.001
				3rd Week			
3	Pap 100/100 H_2O	60.25	1.00	97.215	0.964	0.42	0.001
	Pap 20/80 lime juice	54.74	0.40	97.432	1.627	0.54	0.001
	Pap40/60 lime juice	51.15	0.60	97.284	1.605	0,41	0.001
	Pap 60/40 lime juice	50.89	0.80	97.231	1.578	0.49	0.001
	Pap 80/20 lime juice	50.61	1.00	97.334	1.415	0.45	0.001
				4th Week			
4	Pap 100/100 H2O	56.63	0 .08	97.866	0.963	0.37	0.001
	Pap 20/80 lime juice	52.34	0.40	97.481	1.608	0.51	0.001
	Pap 40/60 lime juice	50.75	0.60	97.346	1.563	0.49	0.001
	Pap 60/40 lime juice	50.31	0.80	97.354	1.475	0.47	0.001
	Pap 80/20 lime juice	49.73	0.07	97.648	1.221	0.43	0.001
	Control Mean (CM)	62.05	1.05	97.05	1.418	0.477	0.001
	Sample mean (SM)	52.52	0.656	97.22	1.592	0.523	0.001

The pH values (Table 7) of the samples from Day 1 to Day 5 indicate a general trend of decreasing acidity over the five-day period, with variations observed among the different samples. Sample A showed a steady decline in pH, starting from 4.35 on Day 1 and dropping to 3.19 on Day 5. This suggests an increase in acidity over time, likely due to microbial metabolic activity. Sample B exhibited a less consistent pattern. While the pH dropped from 4.13 on Day 1 to 3.17 on Day 3, it increased again on Days 4 and 5, reaching 4.17 and 4.02, respectively. This fluctuation could be influenced by the dynamic microbial interactions and fermentation processes in the sample. Sample C showed a more gradual decline in pH from 4.10 on Day 1 to 3.77 on Day 5. This steady decrease indicates a relatively stable increase in acidity. Sample D experienced a similar but more consistent decline, starting at 4.08 on Day 1 and ending at 3.51 on Day 5, reflecting an overall trend of increasing acidity over time. Sample E also demonstrated a decrease in pH, from 4.08 on Day 1 to 3.23 on Day 5, with the most significant drop occurring between Day 4 and Day 5. The bacteria load of ogi treated with lime juice showed a marked decrease compared to the control, with bacterial counts ranging from 2×10^3 to 33.3×10^3 CFU/g. Lime juice exhibited a bacteriostatic effect, as evidenced by delayed bacterial growth observed after extended incubation. This agrees with findings by recent studies attributing lime's antimicrobial properties to its bioactive compounds, including citric acid, flavonoids, and terpenes (Adepoju *et al*., 2022; Okon *et al*., 2023). These compounds disrupt microbial cell walls, inhibit enzyme activities, and alter cellular metabolism, effectively reducing bacterial proliferation. The bacterial species identified included *Lactobacillus* sp., *Leuconostoc* sp., and *Staphylococcus aureus* in the control, while treated samples also harboured *Corynebacterium* sp., *Flavobacterium* sp., and *Klebsiella* sp.. The presence of lime did not eliminate species diversity but significantly curtailed bacterial growth rates. This is consistent with findings that lime juice primarily exerts bacteriostatic rather than bactericidal effects (Ibrahim *et al*., 2021). Notably, *Lactobacillus* sp. persisted, possibly due to its acid-tolerant nature, underscoring the selective action of lime on microbial communities.

The fungal load of ogi samples preserved with lime juice ranged from 12×10^3 to 29×10^3 CFU/g, with isolates including *Aspergillus niger*, *Fusarium* sp., and *Saccharomyces cerevisiae*. The reduction in fungal load, though significant, was less pronounced

than the bacterial count decrease. This is attributed to fungi's ability to thrive in acidic environments, as supported by recent findings indicating that certain fungi possess adaptive mechanisms to withstand low pH conditions (Chowdhury*et al*., 2023).

Table 6: Organoleptic test

Parameters	Samples				
	A	**B**	**C**	**D**	**E**
Colour					
Very bad					
Bad	√				
Good					
Very good		√			
Excellent			√	√	√
Aroma					
Very bad					
Bad	√				
Good		√			
Very good					
Excellent			√	√	√
Appearance					
Very bad					
Bad	√				
Good		√			√
Very good					
Excellent			√	√	
Taste					
Very bad	√				
Bad					
Good		√			√
Very good					
Excellent			√	√	
Viscousity					
Very bad					
Bad	√				√
Good		√			
Very good					
Excellent			√	√	

Table 7: pH values of sample from day 1 to day 5

Samples	**Day 1**	**Day 2**	**Day 3**	**Day 4**	**Day 5**
A	4.35	4.40	3.34	3.31	3.19
B	4.13	4.10	3.17	4.17	4.02
C	4.10	4.06	3.92	3.87	3.77
D	4.08	4.02	3.78	3.64	3.51
E	4.08	4.00	3.92	3.27	3.23

The antifungal properties of lime juice are linked to its citric acid content and other bioactive compounds that interfere with fungal growth. However, their efficacy is limited against acidophilic fungi, aligning with earlier observations that fungi are less affected by pH changes than bacteria (Frazier *et al*., 2021). A steady decline in moisture content was observed in lime-treated ogi samples over four weeks, contrasting with the control. The reduction in moisture can be attributed to lime juice-induced caking or congealing, which restricts water retention. This phenomenon aligns with the theory that reduced moisture levels delay microbial activity by creating less favourable conditions for growth (Olawale *et al*., 2022). Moisture reduction is a critical factor in food preservation, as it inhibits microbial metabolism and prolongs shelf life. The fat content remained stable in lime-treated samples during the initial weeks but showed a gradual decline in the control after the third week. Microorganisms metabolize fats less readily than carbohydrates, often requiring specific enzymes like lipase for hydrolysis. The stability in fat content in lime-treated samples suggests reduced microbial lipase activity, which can be linked to the antimicrobial properties of lime (Labuza*et al*., 2020).

Protein levels fluctuated in the control and treated samples during the first two weeks but declined steadily in the control thereafter. The presence of fermentable carbohydrates in lime-treated samples likely inhibited proteolytic organisms by promoting acid fermentation, which suppresses protein hydrolysis. This protective effect of lime juice on protein content is in agreement with contemporary

studies emphasizing the interplay between fermentation and protein preservation in acidic environments (Yusuf *et al*., 2023). There were no significant differences in ash and fibre content between treated and control samples. The reduction in mineral and fibre content during ogi production is attributed to processing steps, such as sieving, which removes chaff rich in these components. This observation concurs with reports emphasizing the nutrient losses associated with food processing (Labuza, 2020).

Carbohydrate content remained consistent across all samples. The minimal utilization of carbohydrates by microorganisms in lime-treated samples indicates that the antimicrobial properties of lime limited microbial activity. This aligns with recent findings on the carbohydrate-preserving effects of natural antimicrobial agents in fermented foods (Caceres *et al*., 2021).

Lime juice significantly influenced the sensory properties of ogi. Samples preserved with moderate lime concentrations (C and D) exhibited better aroma, taste, and a more appealing golden-yellow colour. These attributes were attributed to lime's impact on microbial activity, which modulates fermentation processes and affects the release of volatile compounds. Additionally, the reduced viscosity in treated samples suggests enzymatic breakdown of starch into simpler sugars, as previously noted in studies on fermented foods (Eliasson and Larsson, 2021).

Lime-treated samples demonstrated a significant decrease in pH, driven by the production of organic acids such as lactic, acetic, and propionic acids during microbial fermentation. The low pH environment inhibited non-acid-tolerant organisms, emphasizing lime's dual role in lowering pH and introducing additional antimicrobial agents. This finding corroborates the work of Prescott *et al*. (2002), who highlighted the synergistic effect of pH reduction and antimicrobial compounds in preserving acidic foods.

Conclusion: Lime juice has been found to significantly extend the shelf life of ogi, a Nigerian weaning food, by up to four weeks. This is due to its ability to reduce microbial activity and delay spoilage. The nutritional content of ogi stored with lime juice remained consistent with the control sample, indicating its potential as a natural preservative without compromising the food's quality. The preservation also improved the food's organoleptic properties, including aroma, taste, and texture. This preservation method could offer a cost-effective and nutritionally adequate alternative for Nigerian households, addressing food security challenges and catering to infants and young children.

Declaration of Conflict of Interest: The authors declare no conflict of interest

Data Availability Statement: Data are available upon request from the first author or corresponding author or any of the other authors

REFERENCE

Adepoju, A; Ogunlade, B; Olamide, O (2022).Antimicrobial properties of citrus-based preservatives in fermented foods. *J. Food Preserv. Sci.* 45(3): 345–356.

Caceres, F; Martinez, J; Gomez, R (2021). Natural antimicrobials in food preservation: A review. *Food Res. Int.* 139: 109994.

Chowdhury, N; Ahmad, T; Habib, R (2023). Adaptation of fungi to acidic environments: Implications for food preservation. *Mycolog. Res.* 127: 45–57.

Eliasson, A; Larsson, S (2021).Fermentation dynamics in starchy foods. *J. Food Biochem.* 44(5): 876–889.

Eze, SO; Ogbulie, JN;Adetunji, AO (2022).Antimicrobial and sensory properties of lime juice in food preservation.*Annals of Microbio*. 72(1): 45–56.

Ibrahim, M; Olaniran, AA; Adetunji, CO (2020). Lime potentials as a biopreservative: An alternative to chemical preservatives in fruit juice blends. *Food Res*. 4(6): 1878–1884.

Ibrahim, R; Adeoye, A; Uchenna, M (2021). Lime juice as a bacteriostatic agent in fermented foods. *Afr. J. Micro. Res.* 15(4): 456–468.

Igiebor, FA; Uwuigiaren, NJ (2024). Influence of soil microplastic contamination on maize (*Zea mays*) development and microbial dynamics. *Discover Environ.* 2:122

Jittanit, W; Pichitlamken, J; Setphan, T (2020).Effects of lime juice on microbial load and sensory quality in food preservation. *Int. J. Food Sci. Tech.* 55(4): 1223–1231.

Labuza, T. (2020). The role of moisture and water activity in food preservation. *Annual Rev. Food Sci. Tech.* 11(1): 147–169.

Maduka, N; Igiebor, FA; Elum, O (2022).Microbiological assessment of home-packed children's meal, antibiotic susceptibility and prevalence of intestinal parasites among pupils in selected schools in Benin City, Nigeria. *FUW Trends Sci. Tech. J.* 7(3): 138 150.

Okeke, C; Ijabadeniyi, OA (2021).Microbial dynamics during ogi fermentation and the role of natural preservatives. *Afr. J. Food Sci. Tech.* 13(3): 105–113.

Okon, R; Akpan, E; Udoh, J. (2023). Evaluation of citrus juice in the preservation of traditional fermented foods. *Int. J. Food. Micro.* 365: 109573.

Osarumwense, JO; Igiebor, FA (2018). Assessment of indigenous bacteria from biodiesel effluents contaminated site. *J. Appl. Sci. Environ. Manage*. 22 (2): 157-160.

Prescott, L; Harley, J; Klein, D (2002).*Microbiology of Food and Fermentation*. 11th ed. McGraw-Hill.

Yusuf, H; Mohammed, A; Umar, T (2023). Fermentation-mediated preservation of protein content in acidic foods. *Food Chem.* 384: 132453.