# Antimicrobial Effect of Organic Acids Produced by Selected Strains of Lactic Acid Bacteria during Storage of *Ugba* (Fermented African Oilbean Seeds)

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## ABSTRACT

The antimicrobial effect of organic acids produced by selected strains of lactic acid bacteria during storage of ugba were examined. The objective was to determine changes in pH, titratable acidity, organic-acid profiles and microbiological quality in starter-culture-fermented ugba compared with spontaneously fermented product. The African oil bean seeds (*Pentaclethra macrophylla* Benth) and the *Alchornea laxiflora* Benth leaves (*Akwukwo ugba* – the popular leaves for wrapping *ugba*) were purchased from Urbani main Market, Umuahia - Nigeria. The seeds were identified, sorted, graded and washed in order to remove spoilt seeds, dust and extraneous materials from wholesome seeds prior to processing. The control and LAB inoculated samples were prepared in the laboratory using the traditional and experimental procedures, and stored at ambient temperature range of 26 - 33 $^{o}$C for 6 days and analyzed at 2 days interval. The *ugba* produced using LAB starter cultures and uninoculated samples were subjected to physicochemical and microbiological analysis during storage. Results show that the LAB inoculated and uninoculated *ugba* samples were significantly different ($P<0.05$) in hydrogen ion concentration (pH) and total titratable acidity (TTA) during storage. The temperature range recorded (28-36$^{o}$C) favoured the growth of lactic acid bacteria. The pH of the inoculated *ugba* samples decreased towards acidity and the uninoculated counterpart increased slightly towards alkalinity, ranging within 3.42 – 7.32, while the total titratable acidity decreased, falling within the range of 0.033 – 0.085 as the storage days progressed. Result of organic acid content showed that; formic, acetic, lactic and butyric acids were detected in the experimental samples and significantly ($P<0.05$) increased as storage period progressed. Lactic acid reached 0.48 mg g−1 in sample B (*L. plantarum* and *L. brevis*) at the end of storage, whereas acetic acid in the spontaneous control was 0.13 mg g−1. The microbial quality of *ugba* samples inoculated with LAB starter cultures showed less microbial load when compared with their uninoculated counterparts during storage. The findings of this study have shown in consistent with contemporary evidence, that LAB can provide food bioprotection through organic acids and other antimicrobial metabolites.

**Keywords:** *Ugba*, lactic acid bacteria, starter cultures, organic acid

## INTRODUCTION

Biopreservation, preservation by the use of biological agents, refers to the extension of the shelf-life and improvement of the safety of foods using microorganisms and/or their metabolites (Ross *et al*., 2002), and the successful manufacture of fermented food products relies on the presence, growth, and metabolism of specific microorganisms (Olaoye *et al*., 2018).

Modern technologies in food processing and microbiological food safety standards have reduced but not eliminated the likelihood of food-related illness and product spoilage in industrialized countries. Food spoilage, which is referred to as the damage of the original nutritional value, texture, flavour of the food that eventually render food harmful to people and unsuitable to eat, is one of the concerns in food industry due to

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the contamination by pathogens, which are frequent cause of food borne diseases. In order to achieve improved food safety against such pathogens, food industry makes use of chemical preservatives or physical treatments (e.g. high temperatures). These preservation techniques have many drawbacks which includes the proven toxicity of the chemical preservatives (e.g. nitrites), the alteration of the organoleptic and nutritional properties of foods, and especially recent consumer demands for safe but minimally processed products without additives. To harmonize consumer demands with the necessary safety standards, traditional means of controlling microbial spoilage and safety hazards in foods are being replaced by combinations of innovative technologies that include biological antimicrobial systems such as lactic acid bacteria (LAB) and/or their metabolites (Nath *et al*., 2013). The increasing demand for safe food has increased the interest in replacing chemical additives by natural products, without injuring the host or the environment.

Nowadays, the consumer pays a lot of attention to the relation between food and health. The general public wants to reduce the use of chemical preservatives in food or feed. Instead, consumers require high quality, preservatives free, safe but mildly processed food with extended shelf-life. This is of course not an easy task to solve. In addition, present legislation has restricted the use of some currently accepted preservatives in different foods (Brul and Coote, 1999). Efforts have concentrated on identification and development of protective bacterial cultures with antimicrobial effects against known pathogens and spoilage organisms. Lactic acid bacteria starter cultures have a primary role of rapid production of organic acids which inhibits the growth of unwanted flora and enhances product safety and shelf-life. It has been reported that, LAB growing naturally in foods produce antimicrobial substances such as lactic and acetic acid, diacetyl, acetaldehyde, enzymes, $CO_2$, hydrogen peroxide and bacteriocins (Olaoye *et al.,* 2008; Nath *et al.,* 2014), which is inhibitory to spoilage organisms (Steinkraus, 2002), and contributing to antimicrobial activity (Nath *et al.,* 2014) by acidifying their environment. The antimicrobial effect of organic acids lies in the reduction of pH and in the action of undissociated acid molecules (Podolak *et al*., 1996).

Lactic acid bacteria are one of the foods fermenting organisms and their activities form one of the most widely used biological preservation technique (Ezeama, 2007). LAB secretion of many antimicrobial substances with presumptive antimicrobial effect in foods could be exploited in preventing many foodborne pathogens and spoilage organisms (Olaoye *et al.,* 2011), and also contribute to the organoleptic and textural profile of a food item. The antagonistic effect of LAB is mainly due to a lowering of the pH of the food, to competition for nutrients, and to the production of inhibitory metabolites (Stiles, 1996). The ability of LAB to produce lactic acid that contributes to reduction in the pH of food products is one of the very important factor in their strong antagonistic activities which they exert against many related and unrelated microorganisms (Olaoye and Dodd, 2010; Olaoye *et al*., 2011; Sahnouni *et al*., 2014), leading to extended shelf life of the fermented product. Most LAB are considered as 'generally regarded as safe', GRAS (Silva *et al.,* 2002), and are used to ensure safety and preserve food quality (Olaoye and Dodd, 2010).

Fermented African oil bean seeds (*Pentaclethra macrophylla* Benth), popularly called ugba or ukpaka in Igbo language, is a ready-to-eat food, which is produced locally through a mixed wild bacteria fermentation of the sliced, boiled and soaked seeds. The major problem encountered by the local processors of this product (*ugba*) is the poor keeping quality. The product has a shelf-life of about three (3) days (Okorie and Olasupo, 2013), which is one of the factors limiting its production on a large scale (Chuku, 2012). Antagonistic properties of LAB allied to their safe history of use in traditional fermented food products make them very attractive to be used as biopreservatives (Nath *et al.,* 2014). The current need for biopreservation has renewed the interest in the search for food compatible antimicrobials produced by microorganisms.

Additional information on the antimicrobial activities and ability of each of the lactic acid bacteria to produce organic acids under a variety of conditions, with the objective of assessing their use in the biopreservation of locally fermented food as a result of the generally regarded as safe (GRAS) status of LAB, would augment the knowledge already available concerning specific characteristics of the lactic acid bacteria involved. Therefore, the present study was aimed at investigating the antimicrobial effect of organic acids produced by selected strains of lactic acid bacteria during storage of *ugba.*

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# MATERIALS AND METHODS

## Collection of samples

The African oil bean seeds (*Pentaclethra macrophylla* Benth) and the *Alchornea laxiflora* Benth leaves (*Akwukwo ugba* – the popular leaves for wrapping *ugba*) were purchased from Urbani main Market, Umuahia - Nigeria. The seeds were identified at the Department of Plant Health Management, Michael Okpara University of Agriculture, Umudike. The seeds were sorted, graded and washed in order to remove spoilt seeds, dust and extraneous materials from wholesome seeds prior to processing. The control and LAB inoculated samples were prepared in the laboratory.

## Preparation of LAB inoculums for use as starter culture

Following previous phenotypic and molecular characterization and evaluation of technological properties of the LAB isolates reported by the authors (Ome *et al*., 2026a,b), three suitable lactic acid bacteria (LAB) were selected among the presumptive LAB isolates and used in the production of *ugba* as starter cultures.

Culture inocula of the isolates were prepared by transferring a loopful of the pure culture each into 10 ml sterile distilled water and shaken using vortex machine at 3,000 g for even distribution using the method described by Kabuo (2008) with slight modification. The 10 ml culture inoculum was used to inoculate approximately 100 g of conditioned African oil bean shreds to help initiate fermentation. In this study, pure cultures of *Lactobacillus plantarum, L. brevis* and *L. fermentum* were selected after successively screened and used in combination as starter cultures.

## Laboratory preparation of *ugba* and the application of starter culture

The traditional and experimental procedures of Olaoye *et al.* (2018) of preparing *ugba* were employed in the laboratory to ferment the product (see Figure 1). The processing of the large brown glossy seeds of the African oil bean to obtain '*ugba*' involved the following; 4 kg of raw African oil bean seeds were boiled in an autoclave at a temperature of 110 ºC and a pressure of 15 pounds per square inch (psi) for 1 h to soften the hard brown testa (i.e. shell).

The heating was discontinued and the seeds were removed in batches and dehulled while warm. After dehulling, a local vegetable shredder (called *Nkwoo* in Ibo) - this is a perforated piece of metal which the seed can ran over at a certain angle and come out in shreds, was used to shred the seeds. Then into boiling water in a pot, the shredded African oil bean seeds were added and boiled with stirring at 5 min intervals, for 30 min. The boiled shreds were poured into sterile sieve to drain out the hot liquor.

Sterile water was sprayed on the slices to completely remove hot liquor. Then the shreds were washed 3times with sterile water, drained off the wash water and steeped in distilled water in a pot and covered. The shreds were steeped for 10 h. After the steeping period, the shreds were vigorously stirred and poured into a sterile sieve (which has been autoclaved at temperature 121ºC and pressure of 16psi) to completely drain out the steep water from the shreds.

Ten millilitres (10ml) of the prepared LAB cultures containing (8.3 - 12.5 x $10^{10}$ Cfu/ml) was used to inoculate 100g sterile African oil bean shreds in singles and aseptically wrapped in sterile *Alchornea laxiflora* Benth leaves *(akwukwo ugba*) washed in sterile water and sterilized over a steam and lightly tied. The wrapped samples were kept in warm environment (34 ºC) to initiate fermentation and further left to ferment for 3 days (72 h) at ambient temperature (28 – 32 ºC) to yield '*ugba'. Ugba* sample that was not inoculated with LAB starter cultures served as control. The prepared *ugba* samples were labelled appropriately and stored at ambient temperature range of 26 - 33 ºC for 6 days and analysed at 2 days interval. Samples were taken at periodic interval for analysis. Figure 1 shows the flow diagram of *ugba* production via traditional and laboratory procedures:

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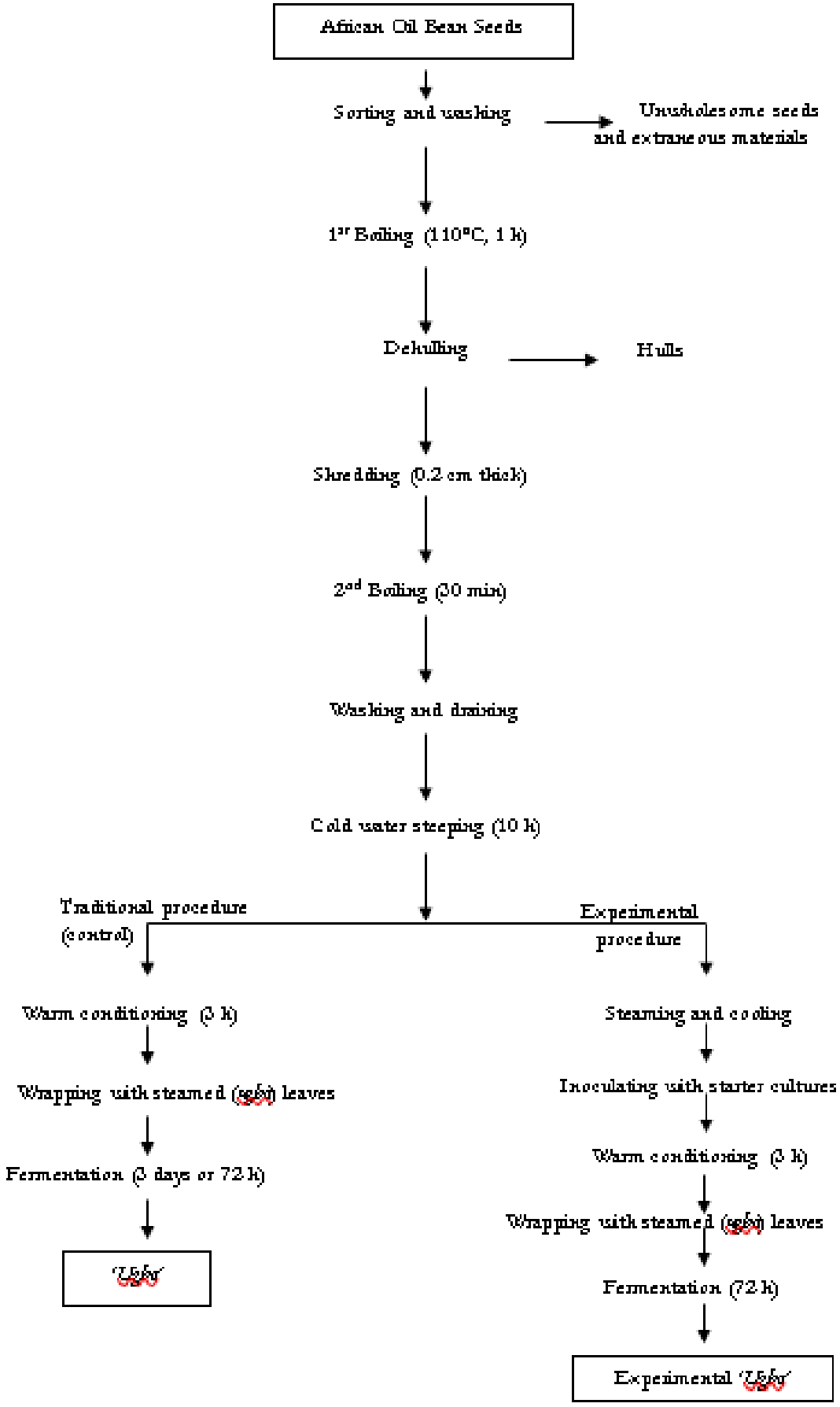


**Figure 1:** Flow diagram of '*Ugba'* production.

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**Determination of acidity:**

**Determination of pH**

A Digital pH meter (model Hanna 211) equipped with glass electrode was used for this purpose. It was used to measure the acidity and alkalinity of the *ugba* samples. It was first standardized using standard buffer solution of pH 4 and 7. The electrode was then rinsed with distilled water before immersing into the samples. Ten grams of the different samples were ground thoroughly in a mortar. This was then suspended in 100ml of distilled water to yield 10% solution. The pH of the suspension was then measured.

**Determination of titratable acidity**

The total titratable acidity of the *ugba* samples were determined by the alkaline titrimetric method described by Eze *et al.* (2014). Approximately 1ml of phenolphthalein indicator was added to a conical flask containing 10ml of *ugba* suspension and titrated against 0.1M NaOH. The mixture was stirred continuously and titration terminated when the colour of the mixture turned pink. The titratable acidity (% lactic acid) was calculated from the formula as follows;

$$\text{TTA (\% lactic acid)} = \frac{\text{Titre value x molarity x 0.09}}{\text{Volume of ugba suspension (ml)}} \times \frac{100}{1}$$

**Determination of organic acids:**

The organic acids were determined using the method of Chaffai *et al*. (2006). The samples were ground finely in ethanol using chilled mortar and pestle. The crude extracts were kept 24 h at 4 °C (in the dark) and then filtered by passing through a glass fibre filter and the residues were washed two times with ethanol. The ethanolic extracts which contain the organic acids were evaporated under low pressure, using rotate evaporator (Büchi). For Thin Layer Chromatography (TLC) analysis, the residues were dissolved in a known volume of EtOH:$H_2O$ (20:2, v/v) mixture.

**Thin layer chromatography (TLC) analysis of organic acids**

Basically, thin layer chromatography (TLC) consists of immobilized solid stationary phase coated thinly onto a glass plate and liquid mobile phase, which flows over the stationary phase. The organic acids were separated by adsorption process, on silica of the order of 0.25 mm thick on glass plate of 20x20 cm (Sigma). The plates were dried in an oven before use at 120 °C for 30 min. This serves to activate the adsorbent. The sample was applied as a narrow band to the stationary phase, 2 cm from the edge by means of a microsyringe. The solvent was removed from the band by gentle heating using of an air blower. Separation took place in a glass tank that contained the developing solvent (mobile phase) to a depth of about 1.5 cm. This was allowed to stand for at least 1 h with a lid over the top of the tank to ensure that the atmosphere within the tank becomes saturated with solvent vapour. After equilibration, the thin layer was placed vertically in the tank so that it stands in the solvent.

The system was kept at constant temperature whilst the development was occurring. Mobile phase, referred to as the eluent, consists of EtOH:$NH_4OH$cc:$H_2O$ mixture (75.5:12.5:12, v/v/v), was then allowed to flow continuously over the stationary phase, resulting in the progressive separation of the organic acids. The plate developed and then was removed from the tank and allowed to dry. A specific organic acid detection was to spray the plate with 0.4% (w/v) bromocresol green in ethanol, in which were added few drops of 0.1 N NaOH, which resulted in yellow spots. The movement of compounds on TLC was characterized by specific $R_f$ values, or retardation factor expressed as $R_f = d_A/d_F$ where $d_A$ is the distance moved by the analyte (organic acid) from the origin and $d_F$ is the distance moved by the solvent front from the origin. Organic acid identification was made on the basis of comparison of the movement of the organic acids with those of reference compounds chromatographed alongside the sample on the TLC plate. The amount of compound present in a given spot was quantified on the basis of spot intensity by means of densitometry.

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**Microbiological analysis of *ugba:***

**Microbial load analysis**

Microbiological analysis were determined using pour plate technique as described by Anyanwu *et al*. (2016) with slight modification in dilution quantity and culture media. Twenty five grams (25g) of the test samples (*ugba*) was crushed in a mortar and 225ml of deionised water was added to make a dilution of $10^{-1}$ of the *ugba* extract. From this dilution, further 10 fold serial dilutions was prepared using a stomacher model 400 (Colworth Seward Medical, London, Uk). One millilitre (1.0 ml) supernatant of various dilutions were inoculated separately into the poured plates of Tryptone Soya Agar (TSA) and Nutrient Agar (CM003, Oxoid) for total aerobic plate count (TAPC), total coliform count (TCC) on MacConkey agar (CM0050, Oxoid), de Man Rogosa Sharpe Agar (MRS) for lactic acid bacteria, Manithol Salt Agar (MSA) for *Staphylococcal* count, *Pseudomonas* count on Nutrient Agar (NA) (at 35-37 °C for 20-24 h) and total fungi count (TFC) was done using 1 mL of the inoculum on Potato Dextrose Agar (PDA) and Sabouraud dextrose agar (at room temperature for 3-7 days). The plates were properly labelled and anaerobically incubated for 24-72 h at 35-37 $^{o}$C. All culture media used were prepared according to manufacturer's instruction. At the end of the incubation, Plates showing between 30 and 300 colonies were counted using the digital illuminated colony counter (Gallenkamp) and results expressed as colony forming units per gram (log Cfu/g) of sample. All counts were done in triplicate and average values were reported.

**Fungal isolates; their characterization and identification**

Fungal isolates were picked with sterile spatula and spread on sterile slides. The properly spread isolates were stained with lactophenol in cotton blue and examined microscopically using various magnifications. The fungi were identified based on their macroscopic cultural characteristics (colonial morphology) and microscopic characteristics on slide culture with reference to standard identification atlas and keys (De-Hoog *et al*., 2007; Tsuneo, 2010).

**Statistical Analysis**

The experimental design used was Completely Randomized Design (CRD). All data obtained which depended on the different treatments with LAB starter cultures and storage periods were subjected to one-way Analysis of Variance (ANOVA) using the statistical software (Statistical Package for the Social Sciences; SPSS) version 22 for Windows; to determine any significant difference at 5% level ($P \geq 0.05$) and differences among means were separated using Duncan's multiple range test and reported as means of three replicates.

## RESULTS AND DISCUSSION

**The pH and titratable acidity of *ugba* samples**

The fermentation was stopped after 72 h and all samples were subjected to six days of storage at ambient temperature. Table 1 shows the temperature, pH and total titratable acidity (TTA) of the ugba samples during storage. The samples differed significantly ($p<0.05$) in pH and TTA. Temperature varied between 28 and 36 °C, providing conditions that supported microbial metabolic activity during storage. The observed differences among treatments are consistent with the established influence of starter culture composition and fermentation conditions on acid production and physicochemical characteristics of plant-based fermented foods (Yang *et al*., 2024).

The pH of inoculated ugba samples decreased towards acidity, whereas the uninoculated control increased towards alkalinity, with values ranging from 3.42–7.32. The increase in alkalinity in the spontaneous product is consistent with the proteolytic nature of alkaline fermentation, in which amino-acid deamination and related reactions can contribute to ammonia formation. Conversely, the progressive acidification of starter-culture treatments is consistent with LAB metabolism and organic-acid production. Recent literature confirms that LAB fermentation of plant matrices can generate organic acids and other antimicrobial metabolites that contribute to inhibition of undesirable microorganisms (Yang *et al*., 2024; Hernández Figueroa *et al*., 2024).

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**Table 1: The pH and titratable acidity of inoculated and uninoculated *ugba* samples during storage**

| Storage period (days) | Samples | Temperature (°C) | pH | TTA (% lactic acid) |
|---|---|---|---|---|
| | A | 36 | $6.19^{c}\pm0.06$ | $0.045^{i}\pm0.002$ |
| 0 | B | 28 | $5.21^{ef}\pm0.11$ | $0.071^{c}\pm0.002$ |
| | C | 31 | $5.36^{e}\pm0.14$ | $0.083^{a}\pm0.003$ |
| | D | 31 | $5.45^{de}\pm0.14$ | $0.076^{b}\pm0.001$ |
| | E | 33 | $5.68^{d}\pm0.06$ | $0.085^{a}\pm0.003$ |
| | | | | |
| 2 | B | 30 | $6.22^{c}\pm0.04$ | $0.067^{de}\pm0.002$ |
| | C | 32 | $5.71^{d}\pm0.31$ | $0.079^{b}\pm0.001$ |
| | D | 30 | $5.17^{ef}\pm0.04$ | $0.068^{d}\pm0.003$ |
| | E | 31 | $7.32^{a}\pm0.01$ | $0.078^{b}\pm0.001$ |
| | | | | |
| 4 | B | 33 | $5.02^{f}\pm0.02$ | $0.052^{g}\pm0.002$ |
| | C | 30 | $4.48^{g}\pm0.02$ | $0.066^{de}\pm0.003$ |
| | D | 28 | $4.01^{h}\pm0.02$ | $0.047^{hi}\pm0.002$ |
| | E | 31 | $7.08^{ab}\pm0.02$ | $0.064^{ef}\pm0.002$ |
| | | | | |
| 6 | B | 31 | $4.51^{g}\pm0.02$ | $0.038^{j}\pm0.002$ |
| | C | 31 | $4.29^{g}\pm0.04$ | $0.049^{h}\pm0.003$ |
| | D | 30 | $3.42^{i}\pm0.02$ | $0.033^{k}\pm0.001$ |
| | E | 32 | $7.01^{b}\pm0.02$ | $0.062^{f}\pm0.002$ |

Values are mean ± standard deviation of triplicate determination with Total titratable acidity (TTA) expressed in (% lactic acid). Means in the same column bearing different superscripts are significantly ($p<0.05$) different.

A: Raw African Oil bean Slices (Unfermented ugba)

B: Sample inoculated with *Lactobacillus plantarum* and *Lactobacillus brevis*

C: Sample inoculated with *Lactobacillus plantarum* and *Lactobacillus fermentum*

D: Sample inoculated with *Lactobacillus brevis* and *Lactobacillus fermentum*

E: Spontaneous fermented *ugba* (Control)

The ability of LAB to lower the pH of the fermented food leads to an inhibition of food spoilage organisms and thus an increase in its shelf life (Ishola and Adebayo-Tayo, 2012). According to Kaban and Kaya (2006); Olaoye and Onilude (2010); Omafuvbe and Enyioha (2011), lowering of pH in food products inoculated with LAB is an important factor in the control of undesirable microorganisms.

In this study, the inoculated treatments generally showed lower pH values than the spontaneous control during storage. However, pH alone cannot be interpreted as proof of antimicrobial activity because LAB may also act through bacteriocins, hydrogen peroxide, competitive nutrient utilization and other metabolites (Hernández Figueroa *et al*., 2024; Putri *et al*., 2024; Chen *et al*., 2025).

The total titratable acidity of *ugba* samples showed a decrease during the storage period with Sample D (*ugba* inoculated with *L. brevis* and *L. fermentum*) having the lowest value (0.033); while Sample E (spontaneous fermented *ugba*) had the highest value (0.085).

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**TLC analysis of organic acid in *ugba* samples as affected by LAB starter cultures during storage**

The thin layer chromatography (TLC) separation of organic acids in ugba samples are presented in Table 2. TLC revealed 4 spots in the ugba extracts, which were identified by comparison of their $R_f$ to those of reference organic acids ($R_{fR}$) as formic ($S_{R1}$, $R_f$ 0.53), acetic ($S_{R2}$, $R_f$ 0.58), lactic ($S_{R3}$, $R_f$ 0.39) and butyric ($S_{R4}$, $R_f$ 0.76) respectively.

Table 3 presents the composition of organic acids in ugba samples during storage. Formic, acetic, lactic and butyric acids were observed in all the experimented ugba samples with lactic acid in sample B (*ugba* inoculated with *L. plantarum* and *L. brevis*) having the highest value (0.48 mg $g^{-1}$) at the end of the storage period, while acetic acid in sample E (control) recorded the least value (0.13 mg $g^{-1}$) and this tallied with the findings of Enujiugha (2003), who reported that acetic acid had the lowest value at the end of 72 h fermentation. Organic acids are the metabolic products of lactic acid bacteria (Olaoye and Ntuen, 2011). Levels and types of organic acids produced during the fermentation process depend on LAB species or strains, culture composition and growth conditions (Anumudu *et al*., 2024; Putri *et al*., 2024). However, it should be stressed that homofermentative LAB can also produce acetic acid but where such is the case, the production is usually very minimal (Bulut *et al.*, 2005; Ammor and Mayo, 2007; Olaoye *et al*., 2008).

The increase in organic acids observed in the inoculated treatments is consistent with current evidence that LAB fermentation of plant-based substrates generates organic acids and other metabolites that can contribute to antimicrobial activity and product functionality (Yang *et al*., 2024; Hernández Figueroa *et al*., 2024).

**Table 2: Thin layer chromatography (TLC) of organic acids from inoculated and uninoculated ugba samples**

| Reference spots | Organic acids | $R_f$ values |
|---|---|---|
| $S_{R1}$ | Formic acid | 0.53 |
| $S_{R2}$ | Acetic acid | 0.58 |
| $S_{R3}$ | Lactic acid | 0.39 |
| $S_{R4}$ | Butyric acid | 0.76 |

$R_f$ = Retardation factor

**Table 3: Organic acid content of inoculated and uninoculated *ugba* samples during storage**

| Storage period (days) | Samples | Organic acid (mg $g^{-1}$) | | | |
|---|---|---|---|---|---|
| | | Formic | Acetic | Lactic | Butyric |
| 0 | B | $0.17^{j}\pm0.01$ | $0.12^{j}\pm0.00$ | $0.29^{gh}\pm0.03$ | $0.35^{gh}\pm0.02$ |
| | C | $0.15^{k}\pm0.00$ | $0.16^{fg}\pm0.01$ | $0.27^{i}\pm0.00$ | $0.31^{i}\pm0.01$ |
| | D | $0.17^{j}\pm0.00$ | $0.15^{gh}\pm0.00$ | $0.30^{fg}\pm0.01$ | $0.36^{fg}\pm0.01$ |
| | E | $0.14^{k}\pm0.01$ | $0.10^{k}\pm0.00$ | $0.25^{j}\pm0.00$ | $0.32^{i}\pm0.00$ |
| | | | | | |
| 2 | B | $0.24^{f}\pm0.00$ | $0.17^{f}\pm0.00$ | $0.34^{e}\pm0.01$ | $0.39^{e}\pm0.01$ |
| | C | $0.21^{hi}\pm0.01$ | $0.23^{c}\pm0.00$ | $0.30^{fg}\pm0.00$ | $0.37^{f}\pm0.00$ |
| | D | $0.23^{fg}\pm0.02$ | $0.19^{e}\pm0.00$ | $0.36^{d}\pm0.00$ | $0.42^{d}\pm0.02$ |
| | E | $0.18^{j}\pm0.00$ | $0.14^{hi}\pm0.01$ | $0.29^{gh}\pm0.00$ | $0.36^{fg}\pm0.00$ |
| | | | | | |
| 4 | B | $0.30^{bc}\pm0.01$ | $0.25^{b}\pm0.00$ | $0.41^{c}\pm0.01$ | $0.44^{bc}\pm0.02$ |

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| | | | | | |
|---|---|---|---|---|---|
| | C | $0.28^{de}$±0.00 | $0.27^{a}$±0.00 | $0.37^{d}$±0.00 | $0.43^{cd}$±0.00 |
| | D | $0.29^{cd}$±0.03 | $0.24^{bc}$±0.01 | $0.43^{b}$±0.03 | $0.47^{a}$±0.01 |
| | E | $0.22^{gh}$±0.01 | $0.17^{f}$±0.00 | $0.31^{f}$±0.00 | $0.39^{e}$±0.03 |
| | | | | | |
| 6 | B | $0.33^{a}$±0.00 | $0.28^{a}$±0.00 | $0.48^{a}$±0.00 | $0.42^{d}$±0.00 |
| | C | $0.27^{e}$±0.02 | $0.25^{b}$±0.00 | $0.42^{bc}$±0.02 | $0.40^{e}$±0.00 |
| | D | $0.31^{b}$±0.00 | $0.21^{d}$±0.04 | $0.47^{a}$±0.00 | $0.45^{b}$±0.01 |
| | E | $0.20^{i}$±0.02 | $0.13^{ij}$±0.00 | $0.28^{hi}$±0.00 | $0.34^{h}$±0.00 |

Results are expressed in mg $g^{-1}$. Values are mean ± standard deviation of triplicate determination.

Means in the same column bearing different superscripts are significantly ($p<0.05$) different.

B: Sample inoculated with *Lactobacillus plantarum* and *Lactobacillus brevis*

C: Sample inoculated with *Lactobacillus plantarum* and *Lactobacillus fermentum*

D: Sample inoculated with *Lactobacillus brevis* and *Lactobacillus fermentum*

E: Spontaneous fermented *ugba* (Control)

All the four organic acids showed significant increases and differences ($p<0.05$) as storage progressed except for formic where Sample B (*ugba* inoculated with *L. plantarum* and *L. brevis*) and sample D (*ugba* inoculated with *L. brevis* and *L. fermentum*), as well as sample C (*L. plantarum* and *L. fermentum*) and sample E (control) that showed no significance ($p>0.05$) on 0 day of storage.

LAB are industrially important because their fermentation metabolism can improve food safety, stability and sensory quality. Current research shows that LAB can produce organic acids, bacteriocins and other bioactive metabolites, with antimicrobial activity depending strongly on strain, substrate and processing conditions (Yang *et al*., 2024; Anumudu *et al*., 2024; Putri *et al*., 2024), all of which can antagonize the growth of many spoilage and pathogenic bacteria in foods (Ammor *et al*., 2006). From the results of this present study, particularly the higher lactic-acid concentration in selected starter-culture treatments, therefore support the potential of defined LAB combinations as protective cultures for *ugba*.

The inhibitory effect observed in this study is most probably associated with the combined action of acidification and other LAB-derived metabolites rather than with lactic acid alone. Recent reviews emphasize that bacteriocins and other antimicrobial metabolites can complement organic-acid-mediated inhibition, while the food matrix can modify their activity (Hernández Figueroa *et al*., 2024; Putri *et al*., 2024; Chen *et al*., 2025). Thus, the higher organic-acid concentrations observed in starter-culture treatments provide a possible basis for the improved microbiological stability observed in those treatments, although direct quantification of individual bacteriocins was outside the scope of this study.

**The microbiological quality of *ugba* samples**

Table 4 shows the mean counts of microorganisms isolated from *ugba* samples from 0 – 6 day of storage at ambient temperature. TAPC shows that Sample E (control) had the highest count (4.66 $\log_{10}$ Cfu/g) while Sample B (*Lactobacillus plantarum* and *Lactobacillus brevis*) had the least (4.32 $\log_{10}$ Cfu/g) at day 0 storage. The TAPC count progressively increased reaching the end (day 6) of storage period. TCC indicates that (<1.0 $\log_{10}$ Cfu/g) count was observed for all the experimented ugba samples from day 0 – 4 storage period. However, Sample E (control) recorded counts (3.15 $\log_{10}$ Cfu/g, 3.44 $\log_{10}$ Cfu/g respectively) on day 4 and day 6 storage period, while Sample D (*Lactobacillus brevis* and *Lactobacillus fermentum*) had the count (2.84 $\log_{10}$ Cfu/g) on day 6 storage period. LAB showed a progressive increase from day 0 – 4 during the storage period with Sample C (*Lactobacillus plantarum* and *Lactobacillus fermentum)* having the highest count (5.42 $\log_{10}$ Cfu/g), while Sample E (control) recorded the least count (5.04 $\log_{10}$ Cfu/g) on day 0. Although, slight

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decline was noticed on day 6 of the storage period. *Pseudomonas* count showed that (<1.0 $\log_{10}$ Cfu/g) count was observed for all the experimented ugba samples from day 0 – 4 storage period. However, Sample D (*Lactobacillus brevis* and *Lactobacillus fermentum*) had the count (2.40 $\log_{10}$ Cfu/g, 2.53 $\log_{10}$ Cfu/g), while Sample E (control) recorded counts (2.63 $\log_{10}$ Cfu/g, 2.71 $\log_{10}$ Cfu/g respectively), on day 4 and day 6 storage period. Staphylococcal count shows that (<1.0 $\log_{10}$ Cfu/g) count was observed for all the experimented ugba samples from day 0 – 4 storage period. Sample E (control) recorded counts (2.62 $\log_{10}$ Cfu/g, 2.65 $\log_{10}$ Cfu/g respectively) on day 4 and day 6 storage period, while Sample D (*Lactobacillus brevis* and *Lactobacillus fermentum*) had the count (2.30 $\log_{10}$ Cfu/g) on day 6 storage period. TFC followed similar trend with *Pseudomonas* as (<1.0 $\log_{10}$ Cfu/g) count was observed for all the experimented ugba samples from day 0 – 4 storage period with Sample D (*Lactobacillus brevis* and *Lactobacillus fermentum*) having the counts (2.20 $\log_{10}$ Cfu/g, 2.30 $\log_{10}$ Cfu/g), while Sample E (control) recorded counts (2.32 $\log_{10}$ Cfu/g, 2.36 $\log_{10}$ Cfu/g), during day 4 and 6 storage period respectively.

**Table 4: Mean count of microbial isolates expressed in ($\log_{10}$ cfu/g) from inoculated and uninoculated *ugba* samples during storage**

| Storage Period (days) | Samples | Microorganisms enumerated | | | | | |
|---|---|---|---|---|---|---|---|
| | | TAPC | TCC | LAB Count | *Pseudomonas* | *Staphylococcal* count | TFC |
| 0 | B | 4.32 | <1.0 | 5.30 | <1.0 | <1.0 | <1.0 |
| | C | 4.52 | <1.0 | 5.42 | <1.0 | <1.0 | <1.0 |
| | D | 4.43 | <1.0 | 5.36 | <1.0 | <1.0 | <1.0 |
| | E | 4.66 | <1.0 | 5.04 | <1.0 | <1.0 | <1.0 |
| | | | | | | | |
| 2 | B | 4.43 | <1.0 | 7.79 | <1.0 | <1.0 | <1.0 |
| | C | 4.59 | <1.0 | 7.83 | <1.0 | <1.0 | <1.0 |
| | D | 4.61 | <1.0 | 7.81 | <1.0 | <1.0 | <1.0 |
| | E | 4.74 | <1.0 | 7.63 | <1.0 | <1.0 | <1.0 |
| | | | | | | | |
| 4 | B | 4.52 | <1.0 | 7.86 | <1.0 | <1.0 | <1.0 |
| | C | 4.71 | <1.0 | 7.90 | <1.0 | <1.0 | <1.0 |
| | D | 4.73 | <1.0 | 7.89 | 2.40 | <1.0 | 2.20 |
| | E | 4.80 | 3.15 | 7.61 | 2.63 | 2.62 | 2.32 |
| | | | | | | | |
| 6 | B | 4.62 | <1.0 | 6.94 | <1.0 | <1.0 | <1.0 |
| | C | 4.76 | <1.0 | 6.99 | <1.0 | <1.0 | <1.0 |
| | D | 4.79 | 2.84 | 7.00 | 2.53 | 2.30 | 2.30 |
| | E | 4.86 | 3.44 | 6.74 | 2.71 | 2.65 | 2.36 |

TAPC = Total Aerobic Plate Count; TCC = Total Coliform Count
LAB = Lactic Acid Bacteria Count; TFC = Total Fungal Count
cfu/g = Colony forming unit per gram
B: Sample inoculated with *Lactobacillus plantarum* and *Lactobacillus brevis*

C: Sample inoculated with *Lactobacillus plantarum* and *Lactobacillus fermentum*

D: Sample inoculated with *Lactobacillus brevis* and *Lactobacillus fermentum*

E: Spontaneous fermented *ugba* (Control)

The results in Table 4 show that the spontaneous control progressively recorded higher bacterial and fungal counts than the starter-culture treatments during storage. This pattern is consistent with recent Nigerian

observations that spontaneously fermented ugba can undergo marked microbiological and sensory changes during ambient storage (Obi *et al*., 2023). Starter cultures can improve process reproducibility by establishing selected microorganisms early in fermentation, thereby reducing ecological opportunity for undesirable organisms. Recent literature also stresses that fermentation safety depends on the interaction between starter culture, food matrix, processing hygiene and storage conditions rather than on fermentation alone (Zannou *et al*., 2023; Anumudu *et al*., 2024). According to De-Muynck *et al.* (2004) their study indicated that the antifungal effect of lactic acid bacteria could not simply be assigned to the low pH, but most probably to the formation and secretion of antifungal organic metabolites or organic acids.

The consistent increase of LAB count throughout the storage period could be attributed to the production of organic acids and especially bacteriocins (Onwuakor *et al.,* 2014). The antimicrobials may have inhibited the growth and the disappearance of some bacterial species during the storage period; which was in agreement with the findings of Ogueke and Aririatu (2004), Eze *et al.* (2014) and Ome *et al*. (2019). The results of this present study are lower than the findings of Anyanwu *et al*. (2016), and Obi and James (2019). The use of starter cultures especially lactic acid bacteria (LAB) has generally been recognized as one major way of ensuring product consistency and to a reasonable extent eliminates the problem of food-borne pathogens (Eman, 2009).

The non-detection of coliforms, *Pseudomonas, staphylococci* and fungi during the early storage period in several treatments may reflect the combined effects of heat treatment, hygienic preparation, competitive LAB growth and antimicrobial metabolites. However, absence by culture-based enumeration could be recorded as non-detection under the analytical conditions used, rather than definitive proof of complete pathogen elimination. Recent research on traditional fermented foods emphasizes the importance of standardized processing, hygienic handling and appropriate microbiological verification to ensure safety (Zannou *et al*., 2023; Anumudu *et al*., 2024). The progressive increase in total aerobic counts during storage also demonstrates that fermentation does not indefinitely prevent microbial growth, highlighting the need for validated storage limits.

## CONCLUSION

This study demonstrated that selected LAB starter cultures altered the physicochemical and microbiological behaviour of ugba during storage. Starter-culture treatments showed progressive acidification and accumulation of organic acids, together with generally lower counts of indicator, spoilage and fungal microorganisms than the spontaneous control. The findings are consistent with contemporary evidence that LAB (*Lactobacillus plantarum, L. brevis* and *L. fermentum*) can provide food bioprotection through organic acids and other antimicrobial metabolites. The results support further development of defined starter cultures for improved consistency and shelf-life of *ugba*, while acknowledging that microbiological safety must be exhibited under controlled processing and storage conditions.

## RECOMMENDATIONS

Further research using other suitable strains of lactic acid bacteria as starter cultures to enhance the production process and keeping quality of *ugba* should be explored.

**Conflict Of Interest**

The authors have no conflict of interest in this work

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