

Impacts of Palm Bunch Ash and Brine Solutions on the Microbial and Organoleptic Properties of “Ugba” (*Pentaclethra macrophyll* Benth)

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Abstract

The impacts of palm bunch ash (PBA) and brine solutions on the microbial and organoleptic properties of ugba were determined. The solutions were prepared from 500 g of PBA and 50 g of NaCl which were dissolved in 1 L water separately. The ugba was produced and divided into 3 equal portions of 500 g. Each portion was cooked in 1 L of water which 50 ml of each solution was added separately; the third portion which is control (CON) was cooked without any solution. The portions were cooled, soaked, washed, wrapped, and fermented for 48 h. Data obtained were subjected to one-way ANOVA using SPSS software of version 21. After 4 weeks of storage, the total fungal counts (TFC) (103 cfu/g) of samples treated with ugba sample treated/cooked in brine solution (BRS₄), ugba sample treated/cooked in PBA solution (PAM₄) and CON₄ were 0.4, 3.7, and 5.4 respectively. Fungal isolates identified were *Rhizopus* sp., *Aspergillus* sp., and yeast, *Penicillium* sp. The ugba samples treated with BRS₄, PAM₄ and CON₄ had total coliform count (TCC) (negligible, 2.3×10^3 and 7.1×10^3 cfu/g), lactic acid bacteria count LABC (negligible, < 10 and 1.7×10^4 cfu/g), and total bacteria count (TBC) (< 10, 3.1×10^4 and 3.8×10^4 cfu/g) respectively. Bacteria isolates identified were *Escherichia coli*, *Salmonella* sp., *Lactobacillus* sp., *Bacillus* sp., *Micrococcus* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Pseudomonas* sp. The overall acceptability showed that ugba sample treated with BRS₄ were liked moderately like CON₄ but significantly different from PAM₄ (approximately 6.0). It showed that brine solution retards microbial growth and improves the organoleptic properties of the ugba after four weeks of storage.

Keywords

Counts, Storage, Agar, Fermentation, Flavor, Sensory, Colonies

Introduction

The African oil bean (*Pentaclethra macrophyll* Benth) is a large perennial tree that is grown mostly in the tropical region of Africa such as Western Africa. It usually grows to a height of 25 m before it starts producing fruits. The leaves are reddish in color when young but slowly change to dark green when maturing [1]. It flowers between March and April after which the pods (brown and woody when matured) open by explosive mechanism, dispersing the seeds and curls up. The glossy brown seeds are the most widely used part of the plant; usually about eight in number are contained in a flattened pod that explodes when fully matured for consumption dispersing the seeds all over the environment where the tree is planted. The African oil bean seed is popularly known as ugba or ukpaka in Igboland (the southern part of Nigeria), apara in Yoruba of Western Nigeria, while the Efik tribe call it ukana [2].

Some studies have been shown to have tried to investigate the microbial status of the ugba to investigate the reason behind its short shelf life. Okorie et al. [3] discovered that the *Bacillus* sp. exhibited the highest potential for ugba fermentation; hence they selected two isolates namely *Bacillus subtilis* and *Bacillus licheniformis* as their starter culture in the fermentation of the ugba. Ugba is a highly perishable food condiment as its shelf life is approximately three days which is very short, implying that the processor must dispose his product within three days of production beyond which time the product goes bad and is thrown away. Thus, there is need to maintain product production consistency by using a known lactic acid bacteria starter culture, unfortunately there is no known starter culture for commercial scale processing of ugba [4]. In addition, Olasupo et al. [5] reported that the major microorganisms involved in the process are *Bacillus* sp. These microorganisms metabolize the protein content of the seeds into free amino acids and ammonia, having undergone a biochemical reaction during the fermentation process known as proteolysis.

Although the fermentation process of the ugba makes it highly nutritive, the lack of hygienic handling of the production process, use of rudimentary equipment that are likely not to be properly sanitized can result to high contamination of the product. Also, emphasis is made on the packaging of the ugba. Oguntoyinbo [6] in his report, speculated that very little attention is placed on the type of packaging used for many traditional foods in West Africa. He went further to state that unhygienic and substandard packaging materials can result in easy contamination by hazardous materials, including biological, physical, and chemical hazard of well-prepared foods during preservation. Ugba is usually wrapped in leaves (in most cases banana leaf), and nylon bags and sold to the public. These packaging materials could be the source of contamination of the product as there is little or no effort around the local processors to properly sanitize the packaging material used for the ugba.

The African oil bean seed generally serves as food condiments which can be eaten alone by garnishing with palm oil, spices and vegetables or it can be mixed with Tapioca to form a Nigerian delicacy popularly known as African salad, can also be used for preparing soups, Nkwobi, sausages and Okporoko sauce. It is usually served in ceremonies and in some eastern regions in Nigeria, served as the first food for visitors/guests [2].

Ugba plays an economic, social, and cultural role among the Igbos in the eastern part of Nigeria as it is mostly served during ceremonies or occasions. It serves as a source of income for the local farmers, it is widely used in preparing local dishes for visitors in most African homes, and it serves as a means of livelihood for most family as they produce and sell in the local markets. Ugba is widely known for its long cooking time and processing. This long cooking and processing invariably affect its nutritional compositions. Several works reported that cooking of ugba reduces its nutritional content [5] and Okoro and Emefieh [7] discovered that fermentation reduced its nutritional components. Thus, the introduction of brine and PBA as a treatment to ugba during its processing may likely increase

their nutritional composition as salt is known to contain some minerals while PBA also contains some minerals [8]. The process of producing ugba which involves long cooking of the seed with the pod and the subsequent fermentation can help to reduce the presence or the microbial load of the product, yet ugba is known to have a short shelf life of about three days unless refrigerated or dehydrated; salt has been used for long as a preservative for food products [9, 10].

PBA, traditionally known as Ngu in eastern Nigeria, is used in place of potash (known traditionally as akanwu by the Igbos, kanwa by the Hausas and kaun by the Yorubas) as food additive and tenderizer [11]. Low quantity of it is advised to be used for cooking, this is because excessive consumption of potash might lead to its accumulation that could cause severe and irreparable damage to the kidney and disrupt normal body functions which may eventually lead to loss of life [12]. PBA produced by burning or ashing, which constitutes about 6.5% by weight of the empty fruit bunch, has high pH and contains varying amounts of other nutrients such as calcium, phosphorus, and magnesium [8]. This makes it a very good source of minerals and its addition to food could bring about an enhancement of the food's mineral components. However, high intake or consumption (at 972 mg/kg body weight) of PBA may be toxic to the body, hence lower concentrations should be used when preparing meals [11].

Brine solution is a mixture of salt and water which usually works to dissolve proteins, turning them into liquids [13]. It helps to entrap water molecules between the proteins thus allowing for a juicier product. Brining is especially used in protein food items such as meat, fish etc. This is because when heat is added to muscle fibers, the individual proteins unwind in a process called denaturing. This subsequently results in moisture loss and shrinkage. Thus, brining helps to counteract the process. This work used a brine solution to pasteurize the ugba to ascertain the effect of the entrapped moisture content on protein content of the final product.

The main objective of this work is to evaluate the impact of PBA and brine solutions on the microbial and organoleptic properties of ugba in the fourth week. Traditional method of processing ugba involves sprinkling of salt on the ugba before fermenting, basically to enhance its taste but there is little or no literature reporting the use of brine solution and PBA in cooking of ugba and its effect on the microbial and organoleptic properties of ugba.

Materials and Method

Materials

Sources of raw materials

The raw uncooked ugba was purchased from a local market at Nkwo-umezeala market, Isiala Mbano LGA, Imo State, Nigeria. The palm bunches were collected from a farm in Umueze, Umuawuchi-owerre, Ehime Mbano, LGA, Imo state where the palm trees were harvested. The banana leaves were gotten from a young banana tree in a farm at Ihiagwa, Owerri, Nigeria. The equipment used for preparing the samples such as pressure pot, gas cooker, bowls, shredder, sieve, muslin cloth

were obtained from Ihiagwa market, Imo state. Other equipment such as measuring cylinder, weighing balance, trays was collected from Food Science and Technology laboratory in Federal University of Technology, Owerri, Nigeria. The whole analysis was carried out at the laboratory of Food Science and Technology in Federal University of Technology, Owerri and VEEPAAT laboratory, Ama, Enugu State, Nigeria.

Preparation of the palm bunch and ash solution

The palm bunches were collected manually and spread on a corrugated zinc and kept under the sun for about three days to dry to enable its proper burning. The dried palm bunch was then burned until it turned to ashes. Later, 500 g were allowed to cool and later was placed inside a large bowl. Afterwards, 1 L water was poured into it and the particles on the water surface were sieved out [8, 11, 12]. This mixture was then filtered continuously until a clear solution, which is the PBA solution was gotten.

Preparation of the brine solution

Then, 50 g of commercial salt was added into 1 L of distilled water and stirred thoroughly until it was completely dissolved [13] to give a clear brine solution.

Preparation of pre-cooked ugba

The traditional method of processing ugba was used to prepare the pre-cooked ugba. The raw ugba was thoroughly washed to remove dirt from the seeds. It was then cooked in a pressure pot for 4 h. The water for its cooking is made to fill up to third fourths of the pressure pot to avoid the water drying out before the end of the cooking period. After four h of cooking, the ugba was left to cool inside the hot water used for its cooking for 30 min before it was drained off. This cooking made it very easy to remove the pods covering the cotyledon. The ugba was then sliced using the shredder to achieve uniform shape and size (Figure 1). The sliced ugba was afterwards divided into three equal portions of 500 g each portion for further processing [2].

Preparation of PAM

The traditional method of preparing ugba was used for its preparation using PBA. 50 ml of the PBA solution was poured into 1 l of water. This was later introduced into the pot containing 500 g of the sliced ugba and it was subsequently cooked for another 2 h. The water used for its cooking was then drained after the ugba was allowed to settle for 30 min [14]. The ugba was then cooled and soaked inside cool water overnight. The soaked ugba was then washed thoroughly (inside the water where it was soaked) by rubbing manually across the palms of the hand continuously for about 30 min. It was then rinsed thoroughly and kept in a sieve for thorough draining of water. After that, the ugba was wrapped inside a banana leaf. It was tied carefully using a part of young palm frond locally known as omu nkwo by the Igbos to avoid any opening that may lead to the contamination of the ugba. It was later placed under the sun for 3 h to raise its temperature. This helps it to achieve the optimum temperature required for the activation of the micro-organisms necessary for its natural fermentation. After a while under the sun, the ugba was

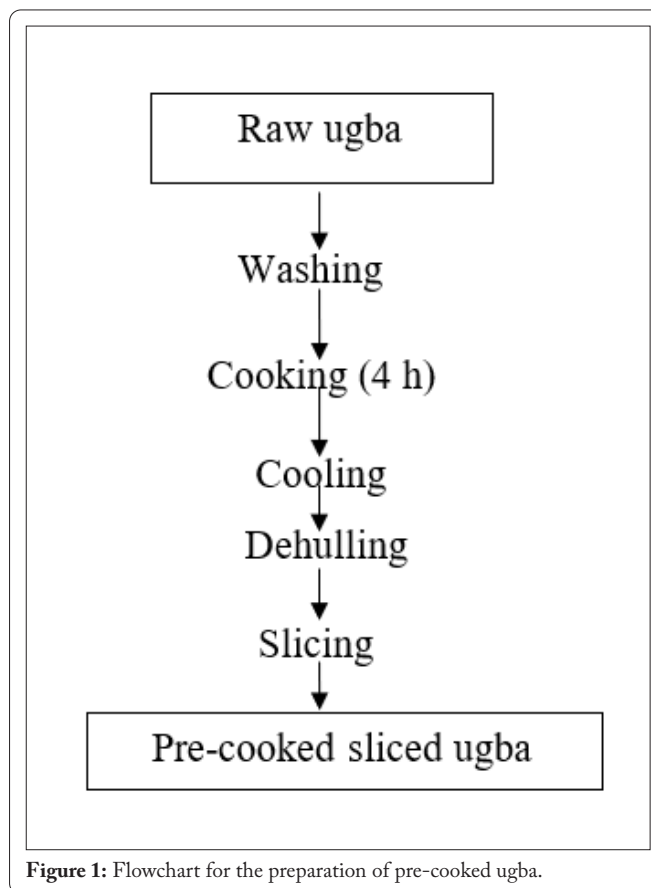


Figure 1: Flowchart for the preparation of pre-cooked ugba.

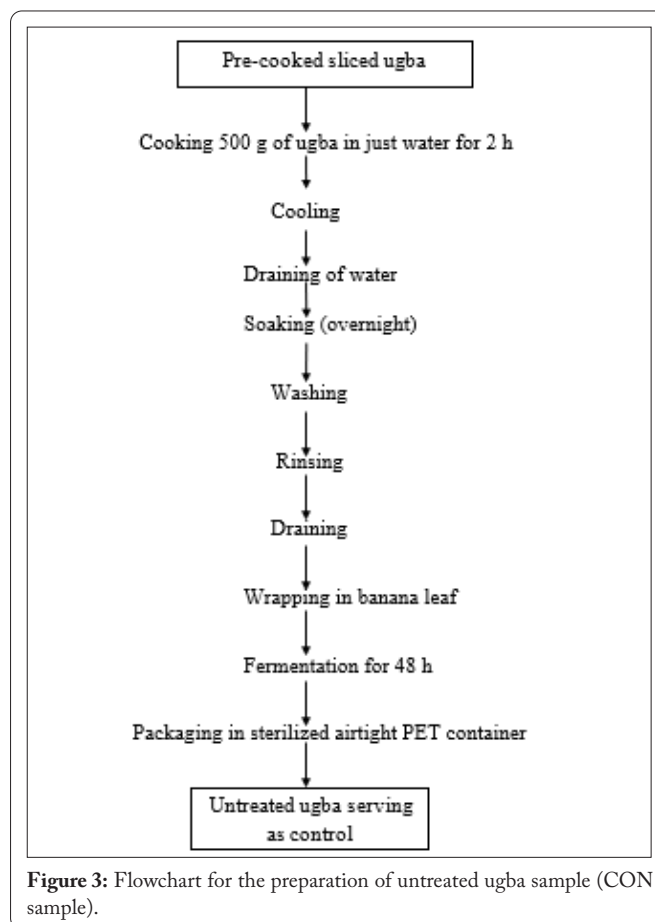
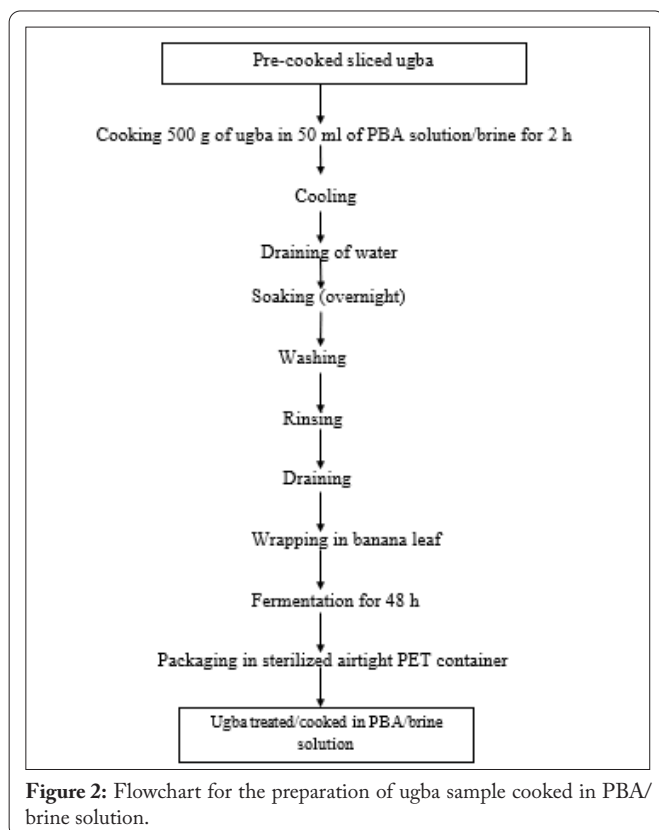
brought in and kept in a warm area and allowed for natural fermentation. The ugba was fermented for 48 h after which it was removed from the banana leaf and placed in a sterilized polyethylene terephthalate (PET) bottle and refrigerated until its analysis in the laboratory (Figure 2).

Preparation of BRS

A 50 ml of brine solution was poured into a portion (500 g) of the ugba. It was also cooked for 2 h. The ugba was allowed to remain in the hot water suspended for about 30 min before it was drained and cooled. The ugba was then soaked overnight. It was then washed thoroughly (inside the water where it was soaked) by rubbing manually across the palms of the hand continuously for about 30 min. It was then rinsed thoroughly and kept in a sieve for thorough draining of water. After that, the ugba was placed inside a banana leaf. It was tied carefully to avoid any opening that may lead to the contamination of the ugba. It was later placed under the sun to raise its temperature. This helps it to achieve the optimum temperature required for the activation of the micro-organisms necessary for its natural fermentation. After a while under the sun, the ugba was brought in and kept in a warm area and allowed for natural fermentation. The ugba was fermented for 48 h after which it was removed from the banana leaf (Figure 2) and placed in a sterilized PET bottle and refrigerated until its analysis in the laboratory [15, 16].

Preparation of the CON sample or untreated ugba

The control sample (that is, the third portion of the ugba which is 500 g) was cooked for the second time (2 h) with



just 1 L of water using the traditional method. The ugba was allowed to remain in the hot water for about 30 min before it was drained and cooled. The ugba was then soaked overnight. It was then washed thoroughly (inside the water where it was soaked) by rubbing manually across the palms of the hand continuously for about 30 min. It was then rinsed thoroughly and kept in a sieve for thorough draining of water. After that, the ugba was wrapped inside a banana leaf. It was tied carefully to avoid any opening that may lead to the contamination of the ugba. It was later placed under the sun to raise its temperature. This helps it to achieve the optimum temperature required for the activation of the micro-organisms necessary for its natural fermentation. After a while under the sun, the ugba was brought in and kept in a warm area and allowed for natural fermentation. The ugba was fermented for 48 h after which it was removed from the banana leaf and placed in a sterilized PET bottle and refrigerated until its analysis in the laboratory (Figure 3 and figure 4).

Methods

Microbial analysis of ugba

Microbial analysis was done to isolate, characterize, identify, and count total bacteria and fungi that may be present in the samples.

Isolation of bacteria from ugba

Bacteriological analyses were carried out on the ugba samples to assess the bacteria isolates. Two grams of the fermented ugba sample were aseptically dissolved in 20 ml distilled water (10% w/v) in a laminar flow (model number). The liquid sample was cultured at 30 °C for 24 h and serially diluted by transferring 1 ml into 9 ml peptone water, and subsequently



until 10 - 5 dilution was attained. One ml of 10 - 5 dilution was spread-plated on nutrient agar (NA) plates and cultured at 30 °C for 24 h. Then, detectible colonies were sub-cultured several times on fresh NA plates and enumerated as described by Cheesbrough [17, 18] and Oyeleke and Istifanus [19, 20].

Determination of the bacterial count in the ugba samples

Fermented sample (ugba) was taken aseptically from traditionally fermented oil bean by using the method of Cowan et al. [21]. One gram of the sample was thoroughly mashed with laboratory pestle and mortar and mixed with 9 ml of normal saline water as diluents in a McCartney bottle and the content

was thoroughly shaken. Subsequent serial dilutions (10 - 2, 10 - 3, 10 - 4, 10 - 5, and 10 - 6) were made from this solution by adding serially 1 ml of solution from preceding concentration to 9 ml of diluents, using sterile syringe. A loopful (0.1 ml) of various dilutions were transferred separately to agar plates using streaking method in triplicates of NA (for bacteria), de Man Rogosa Sharpe (MRS) agar (for lactic acid bacteria), Salmonella Shigella agar (SSA). Bacterial counts were made on nutrient agar plates incubated at 30 °C for 24 h. The total number of colonies developed were counted and expressed as cfu/g of the original sample. Colonies were differentiated based on morphology and counts of different colonial types.

Determination of the fungal count in the ugba samples

Fermented sample (ugba) was taken aseptically from traditionally fermented oil bean by using the method of Cowan et al. [21]. One gram of the sample was thoroughly mashed with laboratory pestle and mortar and mixed with 9 ml of normal saline water as diluents in a McCartney bottle and the content was thoroughly shaken. Subsequent serial dilutions (10 - 2, 10 - 3, 10 - 4, 10 - 5, and 10 - 6) were made from this solution by adding serially 1 ml of solution from preceding concentration to 9 ml of diluents, using sterile syringe. A loopful (0.1 ml) of various dilutions were transferred separately to agar plates using streaking method in triplicates of potato dextrose agar (PDA) with streptomycin for fungi. Fungal plates were incubated at 37 °C for 3 - 5 days and specific counts of MRS agar, plates were incubated at 30 °C for 3 days.

Characterization and identification of bacterial isolates

This was determined using the method of Cowan et al. [21]. Colonies obtained after incubation were sub-cultured on NA which was incubated for 24 h at 30 °C. The cultural characteristics of isolates on the agar plates were observed. The motility of the isolates was examined using hanging drop technique. Gram staining reactions and cell morphology from heat fixed smears were done. The identification procedures for the microorganisms were carried out using methods. The pure cultures of the different organisms isolated were sub-cultured and preserved on agar slants at refrigeration temperature (4 °C). Microscopic examination through gram stain method was done. Biochemical tests such as coagulase, oxidase, indole, urease, methyl red, sugar fermentation, citrate etc., were done as well for the characterization of the bacterial isolate [22].

Characterization and identification of fungal isolates

A mycology atlas was used for identification through a wet mount method. A portion of the mycelium from each culture was dropped into a cotton blue in lacto-phenol on a clean glass slide. A cover slip was used to cover and examine under the microscope (X 40) [22].

Sensory evaluation

The samples were evaluated by 20 untrained panelists who were comprised of students and some staff of the Federal University of Technology, Owerri. They checked for appearance, taste, aroma, texture, and overall acceptability. The scoring was based on a 9 - point hedonic scale ranging from 1 (extremely dislike) to 9 (extremely like) and 5 (neither like nor dislike).

The samples were presented in identical containers coded with 3-digit random alphabets with each sample having a different number. The samples were presented all at once.

Where:

- 9 = Like extremely
- 8 = Like very much
- 7 = Like moderately
- 6 = Like slightly
- 5 = No difference
- 4 = Dislike slightly
- 3 = Dislike moderately
- 2 = Dislike very much
- 1 = Dislike extremely

Meanwhile, necessary precautions were taken to prevent carryover of flavors and overlap during the tasting respectively. This was achieved by ensuring that the panelists rinsed their mouth and washed off their hands properly with water after each stage of evaluation [22].

Statistical analysis

Triplicate data obtained were subjected to statistical analysis using SPSS software of version 23. Mean values were determined, and one-way ANOVA was done as well as fisher's least significant difference (LSD) [23] was used for the separation of the means ($p \leq 0.05$).

Results and Discussion

Microbial properties of fermented ugba treated with different solutions

Effect of solutions of brine and PBA on the TFC of the treated ugba samples

Table 1 shows the results of analysis on the fungal count of fermented ugba samples treated with brine solution and PBA solution respectively. This result reflected the TFC on each sample at one-week intervals for four weeks. Week one recorded the highest TFC 3.3×10^3 cfu/g (ugba treated with brine solution (BRS)), while ugba treated with PBA (PAM) had 4.8×10^3 cfu/g and the control sample (CON) had 8.5×10^3 cfu/g. However, the control sample had the highest TFC (5.4×10^3 cfu/g) after 4 weeks of storage, while the sample treated with brine solution had the lowest TFC (0.4×10^3 cfu/g) at week four. This could be because salt plays a role in the retardation of fungal growth in the sample [24]. Further-

Table 1: Total fungal count ($\times 10^3$ cfu/g) of fermented ugba treated with different solutions.

Weeks	BRS	PAM	CON	Media used
1	3.3	4.8	8.5	SDA
2	1.9	4.1	5.8	SDA
3	< 10	4.3	4.1	SDA
4	0.4	3.7	5.4	SDA

Note: SDA: Sabouraud dextrose agar.

more, the ugba sample treated with PBA solution recorded a steady decline in its fungal count. This suggests that the PBA may have some inhibitory factors that retarded the growth of fungi in the sample, hence a good preservative against fungi; although its TFC is higher than that of brine solution, it is lower than that of the control; hence it is advisable to incorporate it while preparing ugba. Finally, this result shows that salt is a better preservative (especially in fungal control) than PBA. This result is in partial agreement with Obi and James [25] who recorded a decrease in the total count of fungal.

Fungal isolates

The results in table 2 showed the percentage occurrence of fungal isolates from the control sample of the fermented ugba. Their percentage occurrence ranged from 19.05% to 30.16%. It can be deduced that *Rhizopus* sp. has the lowest percentage occurrence (19.05%) while *Penicillium* sp. has the highest percentage occurrence (30.16%). The results in table 3 showed the cultural and morphological characteristics of fungal isolates in the control sample of the fermented ugba. The presence of fungi could be the contamination possibly from the processing environment, handling equipment used, air or water during processing and storage [26]. Hence there is a need to ensure optimum hygiene during processing. According to Azubuine et al. [27], these isolated fungi, especially *Aspergillus* sp., and *Penicillium* sp. have been known to produce mycotoxin, which exposes consumers to food intoxication. Also, the banana leaves which were used to wrap the sample could have been

the source of these fungi invasion [28].

Effect of brine solution and PBA on the bacterial count of the treated ugba samples

Table 4 shows the results obtained on the TCC, lactic acid bacteria and total microbial count of different fermented samples of ugba at one-week interval for four weeks. At week one, the brine solution treated sample had the lowest coliform (4.4×10^3 cfu/g) and lactic acid bacteria (2.1×10^4 cfu/g) count while the untreated sample had the highest coliform (7.9×10^3 cfu/g) and LABC (4.1×10^4 cfu/g). At week two, the TBC of the sample treated with brine solution increased to 4.2×10^4 cfu/g, while that of the untreated sample (control) reduced to 5.8×10^4 cfu/g. At week three, a major decline of TBC was recorded in the sample treated with brine solution, while that of the control increased to 6.9×10^4 cfu/g. Finally, in week four, the sample treated with brine recorded a very insignificant bacteria count (both in coliform and LABC) while that of the control reduced but still had some bacteria present. Additionally, the sample treated with PBA solution recorded a steady decline in its TBC at the four weeks' interval. This result proves that salt may contain some inhibitory properties which retard the growth of bacteria as according to Davidson [29]. Adding salt to foods can also cause microbial cells to undergo osmotic shock, resulting in the loss of water from the cell and thereby causing cell death or retarded growth. In as much as salt retards the growth of microorganisms in food, it favors the growth of the more salt tolerant, beneficial organisms while inhibiting the growth of undesirable spoilage bacteria naturally present in the food [24]. Traditionally, the local ugba producers usually sprinkle salt on the ugba before fermentation, mostly to enhance its taste, but they are oblivious to the fact that salt prolongs the shelf life of the ugba by retarding the growth of harmful bacteria which may be present in ugba, probably introduced during the handling process. Thus, this result suggests that salt is a better preservative than PBA.

Percentage occurrence of bacteria isolates

Table 5 shows the percentage occurrence of bacteria iso-

Table 2: Percentage occurrence of fungal isolates from the CON sample.

Fungal isolates	Positive isolates	Percentage occurrence (%)
<i>Rhizopus</i> sp.	12	19.05
<i>Aspergillus</i> sp.	17	26.98
Yeast	15	23.81
<i>Penicillium</i> sp.	19	30.16
-	63	100

Table 3: Cultural and morphological characteristics of fungal isolates.

Isolate	Culture	Morphology	Probable fungus
1	Bluish grey colonies folding in the centre	Thick walled, no septation, oval spores, occurs in chains and sometimes singly	<i>Penicillium</i> sp.
2	Grey colonies on culture plates	Possess stalk-like conidiospores with large vesicle covered with sterigmata	<i>Aspergillus</i> sp.
3	Black colonies with long antennally branched hyphae with spores	They possess non-septate mycelia, rhizoids and stolon, the sporangium was present	<i>Rhizopus</i> sp.

Table 4: Microbial count (cfu/g) of ugba samples treated with different treatment methods.

Weeks	BRS + TCC + MA	PAM + TCC + MA	CON + TCC + MA	BRS + LABC + NA	PAM + LABC + NA	CON + LABC + NA	BRS + TBC + NA	PAM + TBC + NA	CON + TBC + NA
1	4.4×10^3	6.4×10^3	7.9×10^3	2.1×10^4	2.8×10^4	4.1×10^4	3.9×10^4	5.1×10^4	6.2×10^4
2	4.1×10^3	6.9×10^3	6.3×10^3	1.8×10^4	2.2×10^4	3.6×10^4	4.2×10^4	4.7×10^4	5.8×10^4
3	2.4×10^3	4.5×10^3	6.6×10^3	< 10	2.3×10^4	2.4×10^4	1.5×10^4	3.5×10^4	6.9×10^4
4	NG	2.3×10^3	7.1×10^3	NG	< 10	1.7×10^4	< 10	3.1×10^4	3.8×10^4

Note: MA: MacConkey agar.

lates from the control sample which had *Lactobacillus* sp., and *Bacillus* sp. as the highest occurring bacteria at 19% followed by *Staphylococcus* sp. (18.1%), *Streptococcus* sp. (14.3%), and *Micrococcus* sp. (12.4%). *Salmonella* sp. has the lowest percentage occurrence at 2.9%. *Salmonella* sp. is a food pathogen which is very harmful to human health and its low percentage occurrence reflects that the sample is very fit for consumption. Furthermore, *E. coli* and *Staphylococcus* sp. were among the bacteria isolated. They can cause food borne infections; their low occurrence can be due to the heat treatment given to the sample during its processing. The two highest occurring bacteria isolates (*Lactobacillus* sp., and *Bacillus* sp.) have been found to be the major agents for fermentation as reported by Anyanwu et al. [28]. This result agrees with that of Nwagu et al. [30]. According to Okorie et al. [3], the *Bacillus* sp. isolated in ugba are the major agents of fermentation, hence the fermentation of ugba can be said to be aided by these *Bacillus* sp. that are naturally present in ugba. This also explains the reason for their high occurrence.

Characterization and identification of bacterial isolates

Table 6 shows the isolation, characterization and identification of bacteria which are present in the ugba sample, and

Table 5: Percentage occurrence of bacteria isolates from the CON sample.

Bacterial isolates	Positive isolates	Percentage occurrences (%)
<i>E. coli</i>	4	3.8
<i>Salmonella</i> sp.	3	2.9
<i>Lactobacillus</i> sp.	20	19
<i>Bacillus</i> sp.	20	19
<i>Micrococcus</i> sp.	13	12.4
<i>Proteus</i> sp.	5	4.8
<i>Streptococcus</i> sp.	15	14.3
<i>Staphylococcus</i> sp.	19	18.1
<i>Pseudomonas</i> sp.	6	5
-	105	100

they include *E. coli*, *Salmonella* sp., *Lactobacillus* sp., *Bacillus* sp., *Micrococcus* sp., *Proteus* sp., *Streptococcus* sp., *Staphylococcus* sp., and *Pseudomonas* sp. This result was partly in agreement with that of Okorie et al. [3], Mohammed et al. [31], and Eze et al. [32, 33]. The most predominant bacteria identified from the biochemical test in the control sample were *Bacillus* sp. and *Lactobacillus* species followed by *Staphylococcus* sp., and *Streptococcus* sp. However, from the above result, it could be detected that *Bacillus* sp., and *Lactobacillus* sp. were not the only bacteria present in the samples during the 4 weeks storage. There is evidence that a combination of *Bacillus* sp., *Lactobacillus* sp., *Staphylococcus* sp., and *Streptococcus* sp. [22].

Effect of solutions of brine and PBA on the organoleptic properties of the treated ugba samples

Table 7 shows the mean score of the organoleptic properties on the control sample, sample treated with brine solution and sample treated with PBA at fourth week. The organoleptic properties evaluated include their appearance, taste, aroma, texture, and overall acceptability.

Appearance

This involves the overall physical outlook of the ugba. It includes the shape, size, color, spots, marks, and streaks visible on the ugba. From the result in the table 7, there was no significant ($p > 0.05$) difference amongst the ugba samples in their appearance ranging from 7.05 ± 1.23 to 7.15 ± 1.23 . This shows that all samples retained the standard appearance of ugba after four weeks of storage. Hence, they were liked moderately. This indicated that there was no significant impact on the appearance of the ugba samples caused by the solutions

Table 7: Mean scores of the organoleptic properties of the treated ugba samples.

Sample	Appearance	Taste	Aroma	Texture	Overall acceptability
BRS ₄	7.05 ± 1.23^a	6.10 ± 1.12^a	1.19 ± 1.09^a	6.55 ± 1.15^a	7.15 ± 0.86^a
PAM ₄	7.10 ± 1.25^a	4.55 ± 1.1^b	1.09 ± 1.05^a	5.05 ± 1.32^b	5.95 ± 0.76^b
CON ₄	7.15 ± 1.23^a	4.80 ± 1.5^b	1.11 ± 1.05^a	6.60 ± 1.19^a	6.95 ± 0.83^a
LSD	0.78	0.71	0.67	0.77	0.52

Table 6: Isolation, characterization, and identification of bacteria present in the CON sample of ugba.

Isolates	SH	F	ML	CT	G	GS	O	CH	H ₂ S	VP test	L	GL	X	SU	M	UT	CU	Probable organisms
1	-	±	±	+	±	-	-	-	-	-	±	±	±	±	±	-	-	<i>E. coli</i>
2	-	+	+	+	+	-	-	+	+	-	-	++	-	-	-	-	-	<i>Salmonella</i> sp.
3	+	±	±	-	±	+	-	-	-	-	±	±	±	±	±	-	-	<i>Lactobacillus</i> sp.
4	+	±		+	±	+	+	+	+	+	±	±	±	±	±	+	+	<i>Lactobacillus</i> sp.
5	-	±	-	-	-	+	-	-	-	+	-	±	±	±	±	+	+	<i>Micrococcus</i> sp.
6	+	±	±	-	-	-	-	+	-	-	±	-	±	±	-	+	+	<i>Proteus</i> sp.
7	+	-	-	-	-	+	-	-	-	+	±	+	-	-	-	-	-	<i>Streptococcus</i> sp.
8	-	±	±	+	±	+	-	+	-	+	±	++	±	±	±	-	+	<i>Staphylococcus</i> sp.
9	-	-	±	-	-	-	+	+	+	+	-	±	±	±	±	+	-	<i>Pseudomonas</i> sp.

Note: SH: Starch hydrolysis; F: Fructose; ML: Maltose; CT: Catalase test; G: Galactose; GS: Gram stain; O: Oxidase; CH: Casein hydrolysis; H₂S: Hydrogen sulphide; VP test: Voges proskauer test; L: Lactose; GL: Glucose; X: Xylose; SU: Sucrose; M: Mannitol; UT: Urea test; CU: Citrate Utilisation; +: Positive test; -: Negative test; ±: Acid production; ++: Acid and Gas production.

of PBA and salt. There could be other factors that made the panelist like the samples moderately (approximately 7.0).

Taste

This is the measure of the distinctive flavor of the ugba using the sense of tongue where the taste buds are situated. This analysis will help to determine if the ugba has a sweet, salty, bitter, and sour or umami taste. This ensures that the ugba conforms to the standard taste quality that is generally acceptable especially to the final consumer. The values for taste ranged from 4.55 ± 1.1 to 6.10 ± 1.12 with the sample treated with brine had 6.10 and the sample treated with PBA solution has the least taste value at 4.55 (Table 7). This shows that the sample treated with brine solution was liked slightly in taste, while that of PBA was neither liked nor disliked (approximately 5.0). Hence, salt improved the taste of ugba as suggested by Nzelu [34].

Aroma

This is the measure of the distinctive, pervasive smell associated with fermented ugba. According to Ogueke et al. (2010), the typical aroma of ugba is due to the various volatile compounds produced by the fermenting microorganisms in the course of their metabolism. The determination of the ugba aroma with the use of nose is important as it's aimed at ensuring that the ugba conforms to the standard aroma quality that is generally acceptable, especially to the final consumer. There was no significant ($p > 0.05$) difference amongst the control sample and the sample treated with brine solution at 1.11 ± 1.05 and 1.19 ± 1.09 respectively. This shows that they both gave out almost the same aroma when perceived by the panelists and were disliked extremely.

Texture

Food texture is defined as those properties of a food that are sensed by touch in the mouth and with the hands [35]. Raw ugba is usually hard but becomes softer when fermented. Okoro and Emefieh [7] suggested that fermentation influences the texture of ugba as it softens the cotyledon. This therefore explains why ugba becomes softer upon fermentation. In this research work, the 9-point hedonic scale was used for sensory evaluation. The texture of the samples ranged from 5.05 ± 1.32 to 6.55 ± 1.15 , with the sample treated with PBA having the least value on texture. This can be because of the palm ash being a tenderizer [11]. Hence, it softened the ugba after four weeks.

Overall acceptability

Acceptability is a subjective measure based on hedonics (pleasure), which in turn is influenced by the sensory properties of the food [36]. Overall acceptability is the total perception of a consumer about a food product. It encompasses the total sensory qualities of the food product. The result for the overall acceptability showed that the panelists preferred the sample treated with brine solution to the other two samples at 7.15 ± 0.86 , which means that it was liked moderately. Therefore, salts should be used in cooking and processing of ugba to increase its consumer acceptability.

Conclusion

The impact of brine solution and PBA on the microbial and organoleptic properties of ugba showed that both brine and PBA had some significant effect on the microbial and organoleptic properties of the ugba after four weeks of storage. However, they had a significant effect on the bacterial and fungal count of the ugba. They both had the lowest bacterial and fungal count; the sample treated with brine had a very insignificant bacteria count that one can assume that there were no bacteria present in the sample after three weeks. Hence, they are likely to help extend the shelf life of the ugba.

Additionally, they also affected the sensory properties of the ugba. The sample treated with PBA was found to cook faster than the rest; hence it reduces time of cooking and expenditure. However, it affected the texture of the ugba as its texture appeared to be softer than the other samples. The brine also helped to improve the taste of the ugba. In general, samples treated with brine solution appeared to have the most microbiologically and organoleptically acceptable result, as such, salt should be used in processing of ugba.

Recommendations

It is a known fact that ugba takes a long time to cook. This work discovered that PBA helped to cook the ugba faster, therefore it is recommended that PBA be used while cooking ugba to cook it faster, thus saving time and energy. However, it is worthy of note that it will invariably affect its texture.

The samples were not fermented under controlled conditions and as such may have short shelf life. Thus, it is recommended that other works should use controlled fermentation.

It is recommended that further works should be done on this research, emphasizing more on dehydration of ugba treated with brine and PBA respectively, to ascertain their effect on the shelf life of ugba, as they were found to have the lowest bacterial and fungal count. This can eventually make ugba a potential export product which is beneficial to the economy.

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Conflict of Interest

None.

References

1. Enujiugha VN, Akanbi CT. 2005. Compositional changes in African oil bean (*Pentaclethra macrophylla* Benth) seeds during thermal processing. *Pak J Nutr* 4(1): 27-31. <https://doi.org/10.3923/PJN.2005.27.31>
2. Oly-Alawuba N, Anunukem D. 2018. Effect of processing methods on the nutrient and phytochemical properties of African oil-bean seeds (*Pentaclethra macrophylla*), "ugba". *J Food Sci Eng* 8: 191-197. <https://doi.org/10.17265/2159-5828%2F2018.05.001>
3. Okorie PC, Olasupo NA. 2013. Growth and extracellular enzyme production by microorganisms isolated from ugba-an indigenous Nigerian fermented condiment. *Afr J Biotechnol* 12(26): 4158-4167.

4. Okorie CP, Olasupo NA. 2013. Controlled fermentation and preservation of UGBA – an indigenous Nigerian fermented food. *Springer Plus* 2: 470. <https://doi.org/10.1186%2F2193-1801-2-470>
5. Olasupo NA, Okorie CP, Oguntoyinbo FA. 2016. The biotechnology of ugba, a Nigerian traditional fermented food condiment. *Front Microbiol* 7: 1153. <https://doi.org/10.3389/fmicb.2016.01153>
6. Oguntoyinbo FA. 2014. Safety challenges associated with traditional foods of West Africa. *Food Rev Int* 30(4): 338-358. <https://doi.org/10.1080/87559129.2014.940086>
7. Okoro CU, Emezieh CV. 2018. Production of ugba cubes from fermented pentaclethramacrophylla seeds. *Global J Pure Appl Sci* 24(2): 147-151. <https://doi.org/10.4314/gjpas.v24i2.4>
8. Ojeniyi SO, Awanlemhen BE, Adejoro SA. 2010. Soil plant nutrients and maize performance as influenced by oil palm bunch ash plus NPK fertilizer. *J Am Sci* 6(12): 456-460.
9. Onyekachi IA, Ifeanyichukwu ON, Simonyan KJ. 2021. Postharvest processing, packaging and storage of African oil bean seed. *Poljopr Teh* 46(1): 31-46. <https://doi.org/10.5937/PoljTeh2101032O>
10. Ikhuoria EU, Aiwonegbe AE, Okoli P, Idu M. 2008. Characteristics and composition of African oil bean seed (*Pentaclethra macrophylla* Benth). *J Appl Sci* 8(7): 1337. <https://doi.org/10.3923/jas.2008.1337.1339>
11. Okoye JO, Oranefo NO, Okoli AN. 2016. Comparative evaluation of the effects of palm bunch ash and trona on the liver of albino rats. *Afr J Cell Path* 6(4): 21-27.
12. Bankole JK, Ngokere AA, Ajibade OM, Igunbor CM, Eloka CC. 2015. Degenerating effects of potash (Kaun-K₂CO₃) on the kidney: unabated continental challenge to human health in Nigeria. *Ann Biol Res* 6(3): 12-18.
13. Brining 101. Cookshack. [<https://cookshack.com/blogs/recipes/brining-101>] [Accessed March 24, 2024]
14. Mbata T, Orji MU. 2008. Process optimization in the production and preservation of ugba, a Nigerian fermented food. *Int J Microbiol* 4: 2-6.
15. Itaman VO, Nwachukwu E. 2021. Bacteriological and nutritional assessment of fermented maize (ogi) fortified with ugba (*Pentaclethra macrophylla*). *Nigerian J Microbiol* 35(2): 5906-5917.
16. Joye I. 2019. Protein digestibility of cereal products. *Foods* 8(6): 199. <https://doi.org/10.3390/foods8060199>
17. Cheesbrough M. 2006. Composition and Sensory Properties of "Ugba" (Fermented *Pentaclethra macrophylla* Benth), an African Oil Bean Product. Cambridge University Press.
18. Chessbrough M. 2006. Medical Laboratory Manual. Cambridge University Press, London.
19. Oyeleke SB, Istifanus N. 2009. The microbiological effects of hospital wastes on the environment. *Afr J Biotech* 8(22): 6253-6257. <https://doi.org/10.5897/AJB2009.000-9473>
20. Oyeleke GO, Odedeji JO, Ishola AD, Afolabi O. 2014. Phytochemical screening and nutritional evaluation of African oil bean (*Pentaclethra macrophylla*) seeds. *J Environ Sci Toxicol Food Technol* 8(2): 14-17. <https://doi.org/10.9790/2402-08221417>
21. Cowan CL, Steel KJ. 2004. Manual of Identification of medical Bacteria. Cambridge University Press, London, UK.
22. David-Chukwu NP, Onwuka GI, Aji RU, Odom TC, Chukwu MN. 2021. Production, microbiological and organoleptic properties of stored cocoyam-based products. *Int J Nutr Food Sci* 10(6): 134-143. <https://doi.org/10.11648/j.ijnfs.20211006.13>
23. Landau S, Everitt BS. 2004. A Handbook of Statistical Analyses using SPSS. Chapman and Hall/CRC Press LLC, New York, USA.
24. Doyle MP, Beuchat LR, Montville TJ. 2001. Food Microbiology: Fundamentals and Frontiers. ASM Press, Washington.
25. Obi CN, James MU. 2019. Microbiological and nutritional changes associated with the thermal processing and fermentation of African oilbean (*Pentaclethra macrophylla*) seeds for ugba production. *South Asian J Res Microb* 4(1): 1-5. <https://doi.org/10.9734/SA-JRM%2F2019%2FV4I130100>
26. Peter-Ikechukwu AI, Kabuo NO, Alagbaoso SO, Njoku NE, Eluchie CN, et al. 2016. Effect of wrapping materials on physico-chemical and microbiological qualities of fermented melon seed (*Citrullus colocynthis* L.) used as condiment. *Am J Food Sci Techn* 4(1): 14-19.
27. Azubuine CE, Isu NR. 2006. Fungal contamination of fermenting African oil seeds ugba. *Nig J Microbiol* 20: 931-936.
28. Anyanwu NC, Okonkwo OL, Iheanacho CN, Ajide B. 2016. Microbiological and nutritional qualities of fermented ugba (*Pentaclethra macrophylla*, Benth) sold in Mbaise, Imo State, Nigeria. *Ann Res Rev Biol* 9(4): 1-8. <https://doi.org/10.9734/ARRB/2016/23610>
29. Davidson PM. 2001. Chemical Preservatives and Natural Antimicrobial Compounds. In Doyle MP, Buchanan RL (eds) Food microbiology, Fundamentals and Frontiers. ASM Press, Washington, pp 765-801.
30. Nwagu TN, Orji MU, Nwobodo I, Nwobodo HA. 2011. Mixed microbial flora as starter culture for production of ugba from African oil bean seed. *Asian J Biol Sci* 4(1): 62-69. <https://doi.org/10.3923/AJBS.2011.62.69>
31. Mohammed A, Muhammad M, Sulaiman M. 2020. Bacteriology and proximate composition of Ugba from minna market after prolonged fermentation. *Bima J Sci Technol* 4(1): 189-197.
32. Eze C. 2018. African Oil Bean Seed: 20 Amazing Benefits of *Ukpa-ka/Ugba* you Never Knew. [<https://buzznigeria.com/african-oil-bean-seed-20-health-benefits/>] [Accessed March 24, 2024]
33. Eze VC, Onwuakor CE, Ukeka E. 2014. Proximate composition, biochemical and microbiological changes associated with fermenting African oil bean (*Pentaclethra macrophylla* Benth) seeds. *Am J Microb Res* 2(5): 138-142. <https://doi.org/10.12691/ajmr-2-5-3>
34. Nzelu IC. 2010. Effect of maturity, cooking, salt and fermentation time on the quality of African oil bean (*Pentaclethra macrophylla*) slices: a survey. *Nig Food J* 28(1): 1-9. <https://doi.org/10.4314/nifoj.v28i1.57445>
35. Dahl WJ. 2010. Modifying food texture for the older adult: FSHN10-08/FS156, 7/2010". *EDIS* 2010: 1-3. <https://doi.org/10.32473/edis-fs156-2010>
36. Murray JM, Baxter IA. 2003. Sensory evaluation: food acceptability and sensory evaluation. In Benjamin C (ed) Encyclopedia of Food Sciences and Nutrition. Academic Press, pp 5130-5136.