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Research Article

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Impacts of Palm Bunch Ash and Brine Solutions on the Nutritional and Anti-nutritional Compositions 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. Half of each solution was added separately to each portion, which was cooked in 1 L of water. The third portion, known as the control (CON), was cooked in the absence of any solution. The pieces of ugba underwent a 48 h fermentation process after being cooled, soaked, cleaned, and wrapped. The SPSS software version 23 was used to perform a one-way ANOVA on the acquired data. The proximate compositions of the four ugba samples were: moisture, protein, and ash contents (%) showed significant impacts amongst samples (32.36 ugba sample treated/cooked in brine solution at week 4 (BRS_4); 38.86 ugba sample treated/cooked in PBA solution at week 4 (PAM_4); 47.68 CON_4); (15.69 BRS_4; 12.22 PAM_4; 8.74 CON sample at week 4 (CON_4)); and (3.08 BRS_4; 2.65 PAM_4; 4.01 CON sample at week 4 (CON_4)) respectively. The following vitamin contents were measured in mg/100 g: B_1 (0.043 BRS_4; 0.053 PAM_4; 0.979 CON_4); B_2 (0.078 BRS_4; 0.072 PAM_4; 0.105 CON_4); and B_3 (0.088 BRS_4; 0.091 PAM_4; 0.099 CON_4). The samples exhibit the following anti-nutritional composition (mg/100 g): phytates (1.30 BRS_4; 1.15 PAM_4; 0.64 CON_4), oxalates (8.77 BRS_4; 7.16 PAM_4; 3.72 CON_4), tannins (2.70 BRS_4; 1.88 PAM_4; 1.69 CON_4), and hydrogen cyanide (HCN) (1.93 BRS_4; 1.57 PAM_4; 1.32 CON_4). It was discovered that the nutritional and anti-nutritional compositions of ugba were affected by PBA and brine solutions.

Keywords

Protein, Ash, Moisture, Vitamin B, Tannins, Oxalates, Phytates

Introduction

Pantaclethra macrophyll Benth, also known as the African oil bean, is a tropical tree belonging to the Leguminosae (Mimosoideae) family. It is widely grown in the tropical regions of Africa, particularly in southern West Africa, where it typically grows as a wild tree because no one has been found to cultivate it on their farms [1]. According to Eze [2], the African oil bean can reach heights of 21 meters and depths of about 6 meters. The tree has an open crown to let light pass through its canopy and is low branched with wide buttresses at the base [3]. The woody brown, flattened pod that contains the seeds is glossy brown in color. When the pod explodes at maturity, the seeds inside, which typically come in four pairs per pod, are dispersed throughout the surrounding area, and the empty pods curl up [2]. African oil bean seed is a food condiment in Nigeria that is locally fermented and known by different communities as ugba or ukpaka by the Igbos, apara by the Yorubas, and ukana by the Efiks [4].

The unfermented seeds have a bitter flavor and are rich in saponins and hazardous alkaloids. The product is made nutrient-dense, palatable, and non-toxic by the natural fermentation of the seeds, which is still done at home today. Like many other fermented foods from Africa, it is made solely by mixed fermentation using microorganisms from various sources, some of which may still be alive and active at the time of consumption. Different producers use different methods of production, which leads to a non-uniform product with a limited shelf life [5].

According to Okechukwu et al. [6], the African oil bean seed is a great source of energy, protein, amino acids, phosphorus, magnesium, iron, vitamins, calcium, and manganese. After fermentation, the seed contains approximately 30.80% protein, 38.80% total fats, 1.80% crude fiber, 0.12% total ash, 20.8% phosphorus, 11.40% manganese, 8.06% iron, 3.20% calcium, and 0.26% magnesium. African oil bean seeds are typically prepared into culinary condiments by heating the pods to a soft consistency, slicing the seeds, encasing them in leaves, and letting them ferment for approximately 2 days. The beany flavor and potential toxins like paucine that may be present in the seed are reduced by this cooking and fermentation process [2].

Generally used as a food condiment, African oil bean seeds can also be used to make soups, nkwobi, sausages, and okporoko sauce. They can also be eaten on their own by sprinkling them with palm oil, spices, and vegetables. Another option is to combine them with tapioca to create a Nigerian treat called African salad. In certain parts of eastern Nigeria, it is typically served as the first course for guests or visitors during ceremonies [4].

African oil bean seeds have been shown to have numerous health benefits. Regular consumption of processed African oil bean seeds, or ugba, has been shown to lower the risk of cancer growth [1]. These seeds are also used to make local ointments that are used to treat cuts, scratches, and insect bites [7]. Since they are a good source of oil, businesses can use them to make candles, soaps, and cooking oil. In addition to being used for decorations and bead-making, the pods are used as firewood for cooking [8].

Ugba is well recognized for taking a long time to prepare and cook. Its nutritional compositions are always impacted by this lengthy cooking and processing. Numerous studies have indicated that cooking ugba diminishes its nutritional value [9], while Okoro and Emefieh [10] found that fermentation diminished its nutritional elements. Given that salt and PBA are both known to contain certain minerals, adding brine and PBA to ugba during processing may therefore enhance the nutritional composition of the product [11]. Although the long cooking of the seed with the pod and the fermentation that follows can help to lessen the product's microbial load, ugba is known to have a short shelf life of three days unless it is refrigerated or dehydrated; salt has long been used as a food product preservative [8].

This work aims to assess the effects of brine solutions and PBA on the nutritional and anti-nutritional factors of ugba in the fourth week. The success and output of this work will open the door for a novel method of processing ugba without significantly reducing its nutritional value. Finally, one traditional method of processing ugba is to sprinkle salt on it before it ferments, primarily to improve its flavor. However, there is little to no literature on the effects of cooking ugba with brine solution and PBA on its nutritional and anti-nutritional qualities.

Materials and Method

Materials

Sources of raw materials

The raw ugba was bought from Nkwo-umezeala market in Isiala Mbano LGA, Imo State, Nigeria. The palm bunches were obtained from the farm where the palm trees were harvested in Umueze, Umuawuchi-owerre, Ehime Mbano, LGA, Imo state (Figure 1, figure 2, figure 3, figure 4 and figure 5). The banana leaves came from a young tree on a farm in Ihiagwa, Owerri, Nigeria. The pressure cooker, gas cooker, bowls, shredder, sieve, and muslin cloth were purchased from the Ihiagwa market in the Imo State. Other tools, including trays, a weighing balance, and a measuring cylinder, were taken from the Food Science and Technology Laboratory in Federal University of Technology Owerri, Nigeria. The entire analysis was conducted at the VEEPAAT laboratory in Ama, Enugu State,



Figure 1: Palm bunch.



Figure 2: PBA.



Figure 3: Salt.



Figure 4: African oil bean plant with the pods.



Figure 5: African oil bean seed.

Nigeria, and the Food Science and Technology laboratory at the Federal University of Technology, Owerri.

Preparation of the palm bunch and ash solution

The palm bunches were manually gathered, laid out on a corrugated zinc surface, and allowed to dry in the sun for approximately three days in order to facilitate proper burning. The bunch of dried palms was burned until it was reduced to ashes. 500 g were subsequently allowed to cool before being put inside a sizable bowl. 1 L of water was added, and the particles floating on the surface were removed using a sieve [11-13]. The PBA solution was obtained by continuously filtering this mixture until a clear solution was obtained (Figure 1, figure 2 and figure 3).

Preparation of the brine solution

1 L of water was heated and cooled. Then, 50 g of commercial salt was added into the cooled water and stirred thoroughly until it was completely dissolved [14] to give a clear brine solution (Figure 3).

Preparation of pre-cooked ugba

The pre-cooked ugba was made using conventional processing technique. To clean the dirt from the seeds, the raw ugba was thoroughly cleaned. After that, it was pressure-cooked for 4 h. To prevent the water from drying out before the cooking time is up; make sure the water for the cooking fills up to three fourths of the pressure pot. The ugba was cooked for 4 h and then allowed to cool for half an h inside the hot water before being drained. The hulls covering the cotyledon were very easy to remove because of this cooking. After that, the ugba was cut into uniform sizes and shaped using the shredder (Figure 5 and figure 6). For additional processing, the sliced ugba was then divided into three equal portions, each weighing 500 g [4].

Preparation of ugba cooked in PBA solution

The preparation of ugba was done according to tradition, with PBA. In 1 L of water, 50 ml of the PBA solution were added. This was then added to 500 g of sliced ugba, and the mixture was cooked for an additional 2 h. The ugba was then left to settle for half an h before the water used to cook it was drained [5]. After cooling, the ugba was immersed in cool water for the entire night. After that, the soaked ugba was thoroughly cleaned (inside the soaking water) by repeatedly rubbing the palms of the hand with a handsaw for roughly half an h. After giving it a thorough rinse, it was placed in a sieve to allow the water to drain completely. Subsequently, a banana leaf was used to encase the ugba. It was carefully tied with a section of young palm frond that the Igbo people locally refer to as omu nkwo in order to prevent any openings that could allow the ugba to become contaminated. Later, to increase its temperature, it was exposed to the sun. According to Ju et al. [15], this aids in achieving the ideal temperature needed to activate the microorganisms required for its spontaneous fermentation. The ugba was brought inside and kept in a warm place for natural fermentation after being exposed to the sun for some time. After the ugba had fermented for 48 h, it was taken out of the banana leaf, put in a sterile PET bottle, and chilled until it was analyzed in a lab (Figure 7).

Preparation of ugba cooked in brine solution

A portion of the ugba (500 g) was filled with 50 ml of brine solution. Additionally, it was cooked for 2 h. Before being drained and cooled, the ugba was left in the hot water for roughly 30 min. After that, the ugba was submerged in water overnight. After that, it was properly cleaned (inside the

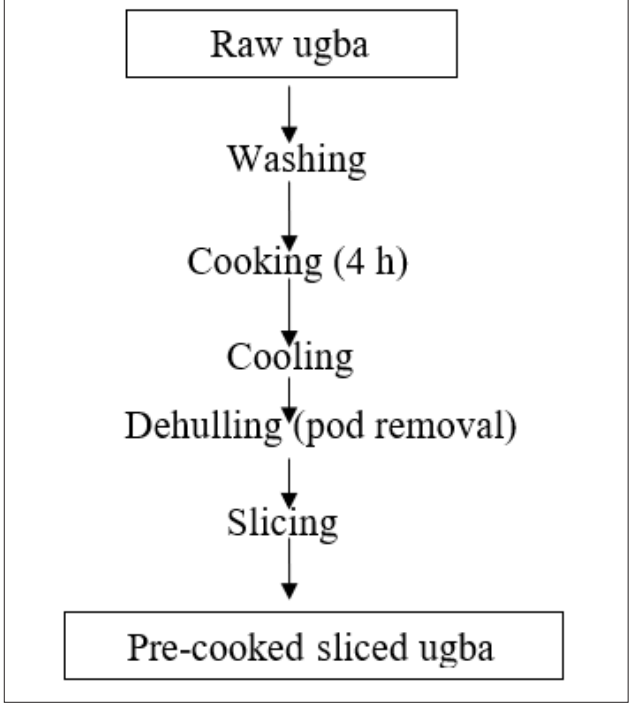

Figure 6: Flowchart showing how to prepare ugba in advance.

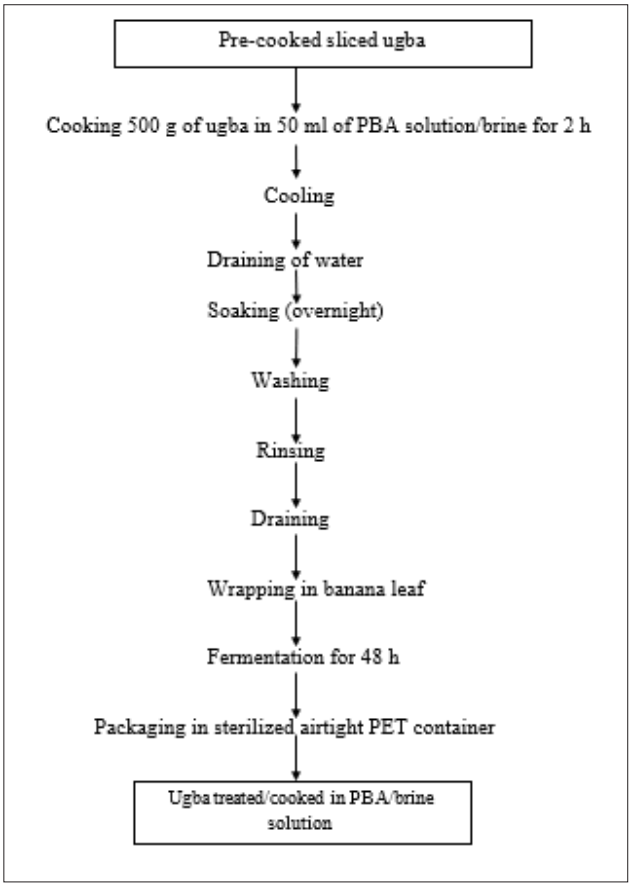

Figure 7: Schematic diagram for preparing ugba samples cooked in a brine/PBA mixture.

soaking water) by repeatedly rubbing the hand's palms with a manual tool for roughly half an h. After giving it a good rinse, it was placed in a sieve to allow the water to drain completely. Subsequently, the ugba was inserted into a banana leaf. It was knotted with extreme caution to seal off any gaps that might allow the ugba to get contaminated. Afterwards, it was exposed to the sun to increase its temperature. This aids in achieving the ideal temperature needed to activate the microorganisms essential for the fermentation process that occurs naturally. The ugba was taken inside and placed in a warm area to allow natural fermentation after being exposed to the sun for some time. Following a 48 h fermentation period, the ugba was taken out of the banana leaf (Figure 7), put in a sterile PET bottle, and chilled until it was analyzed in a lab [3].

Preparation of the CON sample or untreated ugba

Using the traditional method, the CON sample (the third portion of the ugba, weighing 500 g) was cooked for a second time, for 2 h, using only 1 L of water. Before being drained and cooled, the ugba was left in the hot water for roughly 30 min. After that, the ugba was submerged in water overnight. After that, it was properly cleaned (inside the soaking water) by repeatedly rubbing the palms of the hand with a hand tool for about 30 min. After giving it a good rinse, it was placed in a sieve to allow the water to drain completely. The ugba was then enclosed in a banana leaf. It was knotted with extreme caution to seal off any gaps that might allow the ugba to get contaminated. Afterwards, it was exposed to the sun to increase its temperature. This aids in achieving the ideal temperature needed to activate the microorganisms essential for the fermentation process that occurs naturally. The ugba was taken inside and placed in a warm area to allow natural fermentation after being exposed to the sun for some time. Following a 48 h fermentation period, the ugba was taken out of the banana leaf, put in a sterile polyethylene terephthalate bottle, and chilled until it was analyzed in a lab (Figure 8 and figure 9).

Samples analysis

Proximate analysis of ugba samples

To ascertain the moisture, protein, and ash contents of every ugba sample, proximate analysis was utilized. In compliance with the AOAC [16] standard procedures, these were performed in triplicate.

Determination of moisture content

The sample's percentage moisture content was ascertained using the two-stage air-oven method [16]. After weighing 2 g of the ugba sample into dried aluminum crucibles with known weights (W_I and W_2), the crucibles were put in an air oven. For 1 h, the sample was heated to 130 °C and dried. To determine the third weight (W_3), the crucibles were reweighed after being allowed to cool to room temperature. Until a constant weight was achieved, the procedure was repeated. It was determined that the percentage moisture content was:

$$\%\ Moisture\ Content\ = \frac{W_2 - W_3}{W_2 - W_I} \times 100$$

Where: W_I = weight of crucible; W_2 = weight of sample + crucible before drying; W_3 = weight of sample + crucible after drying.

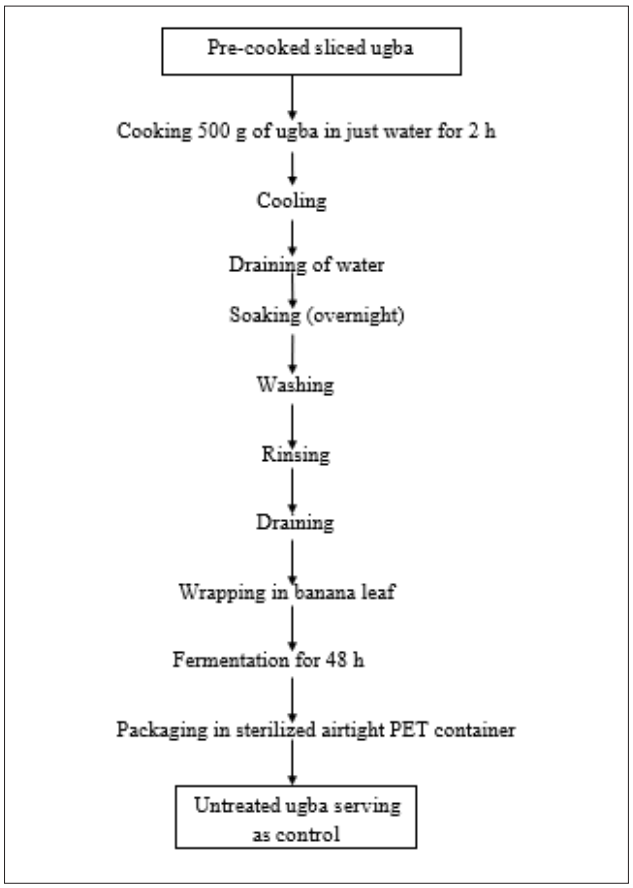

Figure 8: Flow diagram for the untreated ugba sample preparation (CON sample).



Figure 9: Freshly fermented ugba.

Determination of crude protein content

The nitrogen content, as ascertained by the micro-kjedahl method, was multiplied by a conversion factor to obtain the percentage protein content [16]. A sanitized micro-kjedahl digestion flask was filled with 1 g of the sample, which was weighed into an ashless filter paper and carefully wrapped. The sample (ugba) received 20 ml of concentrated sulfuric acid added to it. As a catalyst, a tiny amount of copper sulphate was also added. After that, the flask was set on a digestion mantle and heated slowly until the frothing stopped. After that, it was heated more intensely and shaken occasionally until a clear solution was achieved. After cooling, the digest was poured into a flask containing 100 ml of ammonia-free distilled water and supplemented with anti-bumping granules. After setting up the distillation apparatus, the flask's solution was distilled into 10 ml of 4% boric acid solution that contained three drops of a combination of methyl-red and bromo-cresol green indicator. 50 ml of distillate in total were gathered and titrated against solutions of 0.02 N H_2SO_4. An identical blank sample was also used in the distillation procedure. A color shift to a point indicated the titration's end point. This is how the percentage of crude protein was determined [17]:

$$\%\ Crude\ protein\ = \frac{(V_E - V_b) \times Na \times 0.0014 \times 6.25 \times Vd}{V_{AL} \times M_s}$$

Where: V_E = Titre value for the sample distillate; V_b = Titre value for the blank distillate; V_{AL} = Aliquot of the distillate taken for titration; V_d = Distillate volume obtained; M_s = Mass of test sample; N_a = Normality of acid used (H_2SO_4); 0.0014 = Conversion constant for percentage nitrogen; 6.25 = Conversion constant from percentage nitrogen to protein.

Determination of ash content

2 h were spent at 600 °C in a muffle furnace ashing 1 g of the sample, which was weighed into the crucible in duplicates and burned over a bunsen burner [1]. After being cooled in a desiccator, the crucible was weighed and measured. It was determined that the percentage of ash content was:

$$\%\ Ash\ content = \frac{Weight\ of\ ash}{Weight\ of\ sample} \times 100$$

Determination of vitamin content

The amount of vitamin B in each sample was ascertained through individual analysis. Vitamin B complexes: B_1 (thiamine), B_2 (riboflavin), and B_3 (niacin) are among those assessed. The samples vitamin B content was ascertained through this analysis, which was conducted following the fourth week of fermentation.

Determination of vitamin B_1 (Thiamine)

The AOAC [16] method was used to measure thiamine. Weighing 5 g of each ugba sample, 5 ml of 0.02 M HCl were added to a porcelain mortar until the volume reached seventy ml. For 1 h, the mixture was heated to 50 °C in a water bath with sporadic shaking. The flask's contents were allowed to cool to room temperature after the heating process was completed, and then 100 ml of distilled water was added. It was given several hard shakes before being left to stand for 15 min. Using no. 1 Whatman filter paper, suspensions were filtered. Next, 5 ml of the oxidation solution (a mixture of potassium ferricyanide and NaOH, 1:9 V/V) was added to each of the 5 ml of sample extract that had been pipetted into test tubes with labels. After shaking the mixture, it was given a minute to stand. After adding three drops of hydrogen peroxide solution to each test tube, they were shaken once more. Using an atomic absorption spectrophotometer (AAS Model SP9) with a blank prepared by adding 5 ml of water in place of the sample extract, the absorbance of each sample was measured at 369 nm [18]. There were three copies of the analysis.

$$Vit\ B1\ mg/100\ g = \frac{Abs \times 100 \times 110 \times 1000}{5}$$

Where: Abs = Absorbance; 100 = Volume of extract; 110 = Conversation factor; 5 = Weight of sample taken for extraction.

Determination of vitamin B_2 (Riboflavin)

The AOAC [16] method was followed in performing this analysis of vitamin B_2. For 8 h, 5 g of each UGBA sample were defatted in light petroleum ether at 40 - 60 °C. Each defatted sample was weighed out to make 1 g, which was then placed into a 50 ml conical flask and shaken for 15 min before another 120 ml was added. Whatman no. 1 filter paper was used to filter mixtures. After adding 1 ml of glacial acetic and diluting the mixture to 10 ml with distilled water, the mixture was vigorously stirred. Riboflavin standard solutions were made by precisely weighing out 50, 100, 200, 400, and 600 mg of riboflavin and then dissolving it in 10 ml of distilled water to yield concentrations of 5, 10, 20, 40, and 60 mg/ml, respectively. Ten ml of standard riboflavin solutions and sample extracts were combined with 0.5 ml of 4% $KMnO_4$ solution; the mixture was thoroughly shaken and left to stand for precisely 2 min. This was shaken hard to remove any excess oxygen after adding 0.5 ml of a 3% H_2O_2 solution. A spectrophotometer (AAS Model SP9) was used to measure the absorbance of the yellow-color solution that was produced after turbid or MnO precipitate-containing solutions were centrifuged. Three copies of the analysis were completed. A calibration curve was plotted using the absorption values for various standard riboflavin concentrations, and the linear curve was used to determine the vitamin B_2 content of each sample.

Determination vitamin B_3 (Niacin)

The AOAC [16] method was applied. A 500 ml beaker was filled with 5 g of each ugba sample, 50 ml of 1 N H_2SO_4, and shaken for half an h. Whatman no. 42 filter paper was used to filter the mixture along with three drops of ammonia solution. Ten ml of filtrate were added to 50 ml volumetric flasks using a pipette and potassium cyanide, and 5 ml of 0.02 N H_2SO_4 were added to create a mixture. A spectrophotometer (AAS Model SP9) was used to measure the absorption at 470 nm. The absorbance measured at the same wavelength of 470 nm when a blank was prepared without sample [18]. The following is the calculated niacin content:

$$Vitamins\ B3\ mg/100\ g = \frac{100}{w} \times \frac{au}{as} \times C \times \frac{vf}{va}$$

Where: au = Absorbance of test sample solution; A_s = Absorbance of the blank (CON); V_f = Total volume extract; V_a = Volume of extract titrated; W = Weight of sample analyzed.

Anti-nutritional analysis of ugba samples

The techniques outlined in AOAC [16] were used to identify the anti-nutritional factors.

Determination of tannins

The AOAC [16] method was used to determine the content of tannin. The sample (5 g) was added to 50 ml of distilled water and swirled. The mixture was filtered through Whatman no. 4 grade filter paper after being left to stand for 30 min at 28 °C. A 50 ml volumetric flask was filled with 2 ml of the extract. Similarly, a separate volumetric flask was used as the standard, and it contained 2 ml of standard tannic solution (0.1 mg/ml tannic acid) and 2 ml of distilled water. To each flask, 2.5 ml of saturated sodium carbonate (Na_2CO_3) solution and 1 ml of Folin-C reagent were added, and the volume was brought to 50 ml. The mixture was then thoroughly mixed. Following a 90 min room temperature standing period, the sample was filtered through Whatman no. 4 grade filter paper, and its absorbance was measured at 760 nm in comparison to a reagent blank.

$$Tannins = \frac{Sample\ absorbance \times Standard\ concentration}{Standard\ absorbance \times Weight\ of\ sample} \times 100$$

Determination of oxalate

The titration method was used to ascertain the samples' oxalate content. In a 250 ml volumetric flask suspended in 190 ml of distilled water, 2 g of each sample were added. Each sample received ten ml of HCl solution, and the suspension was digested for 1 h at 100 degrees Celsius. After cooling, the sample was added to the flask until it reached the 250 ml mark. After the sample was filtered, a duplicate portion of 125 ml of the filtrate was measured and added to a beaker along with four drops of methyl red indicator. The concentrated NH_4OH solution was then added drop by drop until the color of the solution changed from pink to yellow. The precipitate containing the ferrous ion was then removed by heating each portion to 90 °C, cooling it, and filtering it. Following another 90 °C heating of the filtrate, 10 ml of a 5% $CaCl_2$ solution was added to each sample, continuously stirring. The samples were cooled and then left over night. After that, the solutions underwent a 5 min, 2500 rpm centrifugation. The precipitates were fully dissolved in 10 ml (20%) of H_2SO_4 after the supernatant was decanted. 200 ml of total filtrate were produced after 2 g of each sample were digested. After the filtrate was heated to almost boiling in aliquots of 125 ml, it was titrated against a 0.05 M standardized $KMNO_4$ solution, producing a pink color that lasted for 30 seconds [1]. The amount of oxalate present in every specimen was computed.

Determination of phytate

Nwosu et al. [19] provided the method used to determine this. After extracting 1 g of each sample with 100 ml of 0.5 N HCl, the excess $FeCl_2$ precipitated the phytic acid in the sample. The precipitate was treated with 2 ml of 2% NaOH to convert it to sodium phytate, and then it was digested using an acid mixture containing 1 ml of concentrated H_2SO_4 and 65% $HClO_4$. Following color development with a molybdate solution, the liberated phosphorous was measured calorimetrically at 520 nm. Phytate as a percentage was computed as follows:

$$\%\ phytate = \frac{AU \times C \times Vt}{Wt \times As \times Va} \times 100$$

Where: A_u = Absorbance of test samples; C = Concentration of standard phytate solutions; V_t = Total volume of extract; W_t = Weight of sample used; A_s = Absorbance of standard phytate solution; V_a = Volume of extract analyzed.

Determination of cyanide

The determination was made using the alkaline titration method as per Onwuka [20] description. A 100 ml sample was steam-distilled into a NaOH solution. Diluted KI solution was used to treat the distillate. After that, a 0.02 M $AgNO_3$ solution was used for titration. When the solution changed from being clear to being permanently turbid, that was the endpoint [1]. In order to calculate the HCN content, 1 ml of 0.02 M $AgNO_3$ was equal to 1.08 mg of HCN.

Determination of saponins

The double solvent extraction gravimetric method was used to determine this. After weighing 2 g of the processed ugba sample, 100 ml of a 20% aqueous ethanol solution, and continuous stirring were incubated at 50 °C for duration of 12 h. Whatman no. 42 filter paper grades were used to filter the mixture. After 30 min, the residue was again extracted using 50 ml of the ethanol solution, and the extracts were combined and weighed. After the combined extract had evaporated to a volume of about 40 ml, it was transferred to a separating funnel and 40 ml of diethyl ether was added. After thorough mixing, there was a partition; the top layer was thrown away, and the lower aqueous layer was extracted again using ether. Next, drop wise addition of NaOH solution was used to lower the pH of the lower layer to 4.5. Using 60 ml and 30 ml portions of n-butanol, the extract's saponin was extracted successively. The combined extract was dried in a water bath in an evaporation dish that had been previously weighed after being rinsed with 5% NaCl solution. To eliminate any remaining solvent, the saponin was dried at 60 °C in a Gallenkamp hot box oven, cooled in a desiccator, and then weighed again. So, the saponin content was determined as follows:

$$\% \, saponin = \frac{W_2 - W_1}{W} \times 100$$

Where: W_1 = Weight of empty evaporation dish; W_2 = Weight of evaporation dish + saponin extract; W = Weight of sample.

Statistical analysis

Version 23 of the SPSS software was used to statistically analyze the obtained triplicate data. After determining the mean values, one-way ANOVA was performed, and Fisher's least significant difference (LSD) [21] was applied to separate the means ($p \leq 0.05$).

Results and Discussion

Proximate composition of different samples of ugba

The proximate compositions of the various ugba samples over a four-week period are displayed in table 1. The moisture content of the four samples varied significantly ($p < 0.05$), with the sample treated with brine solution having the lowest moisture content (32.36 ± 0.93 mg/100 g) and the CON sample at week one having the highest moisture content (51.69 ± 0.02 mg/100 g). In addition, following four weeks of fermentation, the samples' protein content decreased. Following the fourth week, the results revealed that the sample treated with brine solution had the highest protein content at 15.69 ± 0.13 mg/100 g, while the CON sample had the lowest protein content at 8.74 ± 1.09 mg/100 g. The protein content of the samples treated with brine solution at the fourth week and the CON sample at the first week did not differ significantly ($p > 0.05$). According to this, after four weeks, the sample treated with brine solution may have maintained more of its protein content than the samples treated with PBA and the CON sample, respectively. In addition, the CON sample had the highest ash content at 4.01 ± 0.22 mg/100 g in the fourth week, whereas the ugba sample treated with PBA solution had the lowest ash content at 2.65 ± 0.09 mg/100 g. According to this, samples treated with brine solution and PBA solution, respectively, are less likely to contain more mineral compositions than the CON sample. This could be due to the quantity of solutions used in the treatment.

Table 1: Proximate composition of fermented ugba samples treated with different solutions (mg/100 g).

Samples	Moisture	Protein	Ash
CON_0	51.69 ± 0.02^a	16.90 ± 0.86^a	3.07 ± 0.14^b
BRS_4	32.36 ± 0.93^d	15.69 ± 0.13^a	3.08 ± 0.51^b
PAM_4	38.86 ± 0.98^c	12.22 ± 0.19^b	2.65 ± 0.09^b
CON_4	47.68 ± 2.36^b	8.74 ± 1.09^c	4.01 ± 0.22^a
$LSD_{(0.05)}$	2.86	1.27	0.55

Note: abc = significant difference ($p \leq 0.05$); CON_0 = CON at week 1.

Moisture

According to table 1, the ugba samples' moisture contents varied from 51.69 ± 0.02 mg/100 g to 32.36 ± 0.93 mg/100 g. The CON sample had the highest moisture content (47.68 mg/100 g) at week four after fermentation, while the CON sample had the lowest moisture at week one after fermentation. Furthermore, it can be inferred that there was a significant difference ($p < 0.05$) between the samples. Mohammed et al. [22] findings, in contrast, show an increase in moisture at longer fermentation times. As the length of the fermentation process increased, the moisture content dropped. to Yang et al. [23], the moisture content increased as fermentation increased because the microbial and enzymatic hydrolysis of the carbohydrates loosens their structural components and turns them into moisture. However, in this instance, the moisture content decrease suggests that the packaging material used may have played a significant role in the disparity in results, as noted by Kabuo et al. [24]. Moreover, the sample that was subjected to brine solution treatment had the lowest moisture content, measuring 32.36 ± 0.93. This could be the result of salt's preservative action, which lowers food's water activity by drawing water out of it through a process called osmosis [25]. This implies that compared to the other samples, the one treated with brine solution will have a longer shelf life.

Protein

Table 1 indicates that there was a significant ($p < 0.05$) variation between the samples. The ugba samples had protein contents ranging from 8.74 ± 1.09 mg/100 g to 16.90 ± 0.86 mg/100 g. One day after fermentation, the CON sample had the highest protein content, while the CON sample had the lowest protein content. Given that the CON sample's protein content was determined after four weeks, the discrepancy may

have resulted from a different fermentation period. However, this indicated that following the fourth week for the CON sample, the protein content dropped to half its initial value in week one. According to Okoye et al. [12], the fermentation and heat treatment applied to it during processing may have caused this drop in protein content. Furthermore, it is consistent with the findings of Okoro and Emefieh [10], who noted that ugba's protein content decreased following fermentation. However, Okereke and Onunkwo [26] suggested that the decrease in protein content might be the consequence of storage; therefore, more caution should be used when storing ugba to avoid losing its nutritional value. The sample treated with brine solution had the highest protein content compared to the other samples after week four, as can be observed. As a matter of fact, there was no discernible ($p > 0.05$) difference between its protein content at week one and week four compared to the CON group. Given that salt maintained the highest protein content of all the samples in week four, this suggests that salt may have a protein binding property.

Ash

The samples' ash contents varied from 2.65 ± 0.09 mg/100 g to 4.01 ± 0.22 mg/100 g, with the CON sample having the highest ash content after four weeks and the sample treated with PBA solution having the lowest. Since low ash content is a marker or determinant of the sample's mineral composition, it may inevitably have an impact on the sample's mineral content [27]. One possible explanation for the low ash content of the PBA solution-treated sample is that only 50 ml of the solution were used. Moreover, no discernible ($p > 0.05$) difference was found between the sample treated with brine solution at week four and the CON at week one. Therefore, compared to the sample treated with palm ash bunch solution, the sample treated with brine solution is probably more mineral-rich. The findings of Obi and James [28], who observed an increase in ash content ranging from 2.1 ± 0.1 to 2.8 ± 0.1 mg/100 g, were further at odds with this one.

Vitamin B composition of ugba

The B complex vitamins, thiamine (B_1), riboflavin (B_2), and niacin (B_3), are the ones that are tested. Table 2 indicates that the samples treated with brine solution and PBA solution do not differ in a way that is statistically significant ($p > 0.05$); as a result, when ingested, they are expected to provide an equivalent amount of vitamin B. The treated samples' vitamin contents rose with fermentation and at week one was significantly ($p < 0.05$) different from the CON, though they were still lower at week four. Olasupo et al. [9] report that during fermentation, the vitamin B complex tends to decrease. Processors could be to blame for this. This finding contradicted the assertion, though, since after four weeks of fermentation, the vitamin content rose, which is consistent with Okoye et al. [12] research on the impact of fermentation on food's nutritional quality. According to Okoye et al. [12], fermented foods also contain a variety of microorganisms, including lactic acid bacteria, yeast, and mycelia, which aid in changing the raw materials' chemical composition during fermentation and enhancing the food product's nutritional value.

Table 2: Vitamin content of fermented ugba samples treated with different solutions (mg/100 g).

Samples	Vitamin B_1 (Thiamine)	Vitamin B_2 (Riboflavin)	Vitamin B_3 (Niacin)
CON_0	0.034 ± 0.004^{c}	0.050 ± 0.002^{c}	0.051 ± 0.002^{c}
BRS_4	0.043 ± 0.002^{bc}	0.078 ± 0.004^{b}	0.088 ± 0.002^{b}
PAM_4	0.053 ± 0.002^{b}	0.072 ± 0.020^{b}	0.091 ± 0.003^{b}
CON_4	0.079 ± 0.014^{a}	0.105 ± 0.004^{a}	0.099 ± 0.003^{a}
$LSD_{(0.05)}$	0.013	0.02	0.004

Note: abc = significant difference ($p \leq 0.05$).

Thiamine (Vitamin B_1)

Samples containing 0.034 ± 0.004 mg/100 g to 0.079 ± 0.014 mg/100 g were found to contain thiamine. The thiamine content of the CON sample increased significantly from week 1 to week 4. This helps to explain why fermentation raises the thiamine content of ugba. Additionally, there is little ($p < 0.05$) difference between the sample treated with PBA and the sample treated with brine solution. This finding, however, differs from that of Olasupo et al. [9], who found that the fermentation process decreased the amount of vitamin B_1. This variance could result from various processing techniques.

Riboflavin (Vitamin B_2)

Table 2 shows that the vitamin B_2 content of the CON sample at week 4 was 0.105 ± 0.004 mg/100 g, the highest amount, while the vitamin B_2 content of the CON sample at week 1 was 0.050 ± 0.002 mg/100 g. This demonstrates how the amount of vitamin B_2 rose as fermentation time increased. This is consistent with Okoye et al. [12] finding that fermentation raises vitamin content of ugba. Since there was no significant ($p < 0.05$) difference between the samples treated with brine solution and PBA solution, respectively, it can be concluded that when consumed, they both provided the same amount of vitamin B_2.

Niacin (Vitamin B_3)

After four weeks of fermentation, there was an improvement in the amount of Niacin present in ugba. Between week one and week four, the niacin content increased from 0.051 mg/100 g (CON) to 0.099 mg/100 g (CON). Between the two treated samples (one treated with brine solution and the other with PBA solution), there was no discernible ($p < 0.05$) difference.

Anti-nutritional properties of the treated ugba samples and the untreated ugba samples

The various anti-nutritional components of ugba are shown to vary in table 3, with the CON sample at week one having the highest amount of anti-nutrients and the CON sample at week four having the lowest amount. Furthermore, compared to the original CON sample, we can deduce that the treated samples contain fewer anti-nutrients. There are minor but statistically significant differences between the treated samples at ($p < 0.05$). According to Okorie and Olasupo [29], the processing techniques were the cause of the anti-nutrient decrease.

Table 3: Anti nutritional properties of fermented ugba samples treated with different solutions (mg/ 100 g).

Samples	Tannins	Oxalates	Phytates	HCN	Saponin
CON_0	3.84 ± 0.36[a]	12.24 ± 0.57[a]	1.94 ± 0.14[a]	3.85 ± 0.29[a]	Not significant
BRS_4	2.70 ± 0.23[b]	8.77 ± 1.14[b]	1.30 ± 0.07[b]	1.93 ± 0.09[b]	Not significant
PAM_4	1.88 ± 0.13[c]	7.16 ± 0.11[c]	1.15 ± 0.02[b]	1.57 ± 0.14[c]	Not significant
CON_4	1.69 ± 0.04[c]	3.72 ± 0.36[d]	0.64 ± 0.14[c]	1.32 ± 0.05[c]	Not significant
$LSD_{(0.05)}$	0.43	1.25	0.21	0.32	Not significant

Note: abc = significant difference ($p \leq 0.05$).

Tannin

Tannins are plant-based polyphenols that can combine with metal ions and large molecules like polysaccharides and proteins to form complexes. Table 3 shows the range of tannin content: 1.69 ± 0.04 mg/100 g to 3.84 ± 0.36 mg/100 g. Week 4 saw a significant ($p < 0.05$) difference between the CON and brine solution-treated samples, but no significant ($p < 0.05$) difference between the PBA-treated samples and the CON sample. In line with Olasupo et al. [9], who observed that tannin content ugba decreased from 12.58 mg/100 g to 3.65 mg/100 g following fermentation, this was also confirmed. Tannic acid content was found to have decreased following fermentation, which Okoye et al. [12] ascribed to the use of cooking, soaking, and fermentation techniques.

Oxalate

After oxalate and calcium combine, the body is unable to absorb the insoluble calcium oxalate. According to Uzoukwu et al. [1], this could result in hypercalcemia in the renal tubules, which could be fatal. Between 3.72 ± 0.36 mg and 12.24 ± 0.57 mg of oxalate per 100 g, there was a variation in the content. The difference between these samples was statistically significant at ($p < 0.05$). The sample under control had the highest oxalate content during the first stage of fermentation, and the lowest oxalate content during the fourth week of fermentation. It follows that as fermentation increases, ugba's oxalate content drops. The aforementioned outcome was consistent with the findings of Okoye et al. [12], who similarly observed a reduction in the oxalate content during their investigation on ugba. Additionally, this outcome concurs with Okoro and Emefieh [10], who suggested that the processing techniques employed were responsible for the decrease in oxalate content. It is important to highlight that, in contrast to other anti-nutrients in table 3, oxalate values are higher. This is consistent with the findings of Onwuliri et al. [30], who likewise observed a greater oxalate concentration. Uzoukwu et al. [1] had reported that cooking could result in a significant reduction of ugba's total oxalate contents to harmless content. This implies that the other anti-nutrients were lessened by ugba processing than by oxalate.

Phytate

The phytate value of the CON sample was highest at the beginning of fermentation (week one) at 1.94 ± 0.14 mg/100 g, and lowest at week four at 0.64 ± 0.14 mg/100 g (Table 3). With the exception of the CON sample at week four, there was no significant ($p < 0.05$) difference observed between the samples. This finding indicates that the amount of phytate in ugba decreases as fermentation increases; Okoye et al. [12] confirmed that the majority of the phytate in ugba was either lost or removed during processing.

HCN

When consumed in large quantities by monogastric animals, HCN is toxic [13]. The maximum amount of HCN in garri (10 mg/kg) that is advised [32]. The concentration of cyanide in 100 g varied between 1.32 ± 0.05 and 3.85 ± 0.29 mg. In the first week, the CON sample had the highest cyanide content, and in the fourth week, the lowest cyanide content was recorded (Table 3). The samples treated with brine solution at week four and the CON sample at week four differed significantly ($p < 0.05$), but the samples treated with PBA solution at week four and the CON sample at week four did not differ significantly ($p < 0.05$). This result is consistent with that of Onwuliri et al. [30], who also observed that fermentation reduced the amount of cyanide in ugba. HCN may have decreased as a result of the processing techniques used, according to Uzoukwu et al. [1].

Saponin

Protein content in a food sample can be decreased by the formation of saponin-protein complexes [31, 1]. Saponin was of no use because its presence during analysis was not noted. This demonstrated how fermentation and processing totally eliminated the saponin content. According to Okoye et al. [12], processing causes saponins to be lost. It is accurate to state that as a result of the processing techniques our samples underwent, their saponin content was entirely lost (Table 3).

Conclusion

The present study discovered that, following four weeks of storage, the nutritional and anti-nutritional qualities of the ugba were significantly impacted by both brine and PBA The sample treated with brine solution at week four, retained almost the same amount of protein retained by the fresh ugba at week one. This means that cooking ugba with salt could result to retention of the protein contained in ugba, which several works had reported to be lost during its processing. Furthermore, the sample treated with PBA cooked faster than others; this discovery could lead to shorter cooking time of ugba. However, the low ash content recorded by the treated samples could be as a result of quantity (low concentrations at 50 ml) of the solutions used for the ugba.

Recommendations

This research represents a step forward in the quest to find novel ways to preserve the nutritional value of ugba, which is destroyed during processing. Therefore, for additional processing, we advise using a larger volume of brine and PBA solution. The nutritional content of the ugba might be significantly impacted by this. Although it is well known that ugba takes a long time to cook, this study found that using PBA helped the ugba cook more quickly. As a result, it is advised to use PBA when cooking ugba to speed up the cooking process and save time and energy.

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None.

Conflict of Interest

None.

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