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Research Article

EFFECT OF FERMENTATION TIME ON THE NUTRITIONAL, PHYTOCHEMICAL AND MICROBIOLOGICAL QUALITY OF "UKWA BEKEE" (*ARTOCARPUS COMMUNIS)* LEAF EXTRACTS

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ABSTRACT

Objective: The aim of this work was to determine the effect of fermentation time (0, 3, 6, 9, and 12 hrs) on the nutritional, phytochemical, sensory, and microbiological properties of seedless breadfruit (*Artocarpus communis)* leaf of the formulated leaf extract of *A. communis* at different periods of fermentation).

Methods: Freshly harvested seedless breadfruit (*A. communis*) leaves were collected, washed, sliced into sizes of 2-4 mm, weighed and fermented at room temperature at different time intervals (0, 3, 6, 9, and 12 hrs). The fermented leaves were blended, sieved and the extracts were further analyzed to determine the nutritional, phytochemical, microbiological qualities, and sensory evaluation.

Results: Results obtained revealed the following ranges: Moisture (96.62-96.70%), protein (2.48-2.30%), fat (0.56-0.50%), ash (0.29-0.30%), carbohydrate (0.05-0.19%), phytate (6.80-0.81 mg/100 ml), tannin (20.83-16.13 mg/100 ml), saponin (0.18-0.14%), and oxalate (4.10-2.07 mg/100 ml). The phytochemical parameters were carotenoid (0.04-0.07%), flavonoid (1.28-0.66%), alkaloid (0.09-0.38%), phenol (0.48-0.26%). Other nutrients were vitamin C (26.49-18.37 mg/100 ml), vitamin E (0.01-0.03 mg/100 ml), vitamin B_1 (0.46-0.47 mg/100 ml), and vitamin B_2 (0.28-0.57 mg/100 ml) while minerals: Magnesium (3.47-1.48 mg/100 ml), calcium (0.88-0.64 mg/100 ml), iron (0.34-0.21 mg/100 ml), and phosphorous (5.57-0.45 mg/100 ml) were obtained. Microbial load ranged from 1.99 to 1.60×10^5 cfu/ml which showed a decrease with increase in the periods of fermentation. From the microbiological characterization, it was observed that the probable microorganisms belong to *Salmonella* spp., *Shigella* spp., *Bacillus* spp., *Serratia marcescens, Klebsiella* spp., *Pseudomonas aeruginosa, Staphylococcus* spp., and *Escherichia coli.* Sensory scores showed that the unfermented *A. communis* leaf extract was the most preferred by the panelists for overall acceptability (7.53). This could be attributed to the color and aftertaste which had the values of 7.50 and 7.03, respectively.

Conclusion: From the study, it was concluded that the samples fermented for 9 and 3 hrs and had a high nutritional composition and bioactive compounds. It was observed that the color and overall acceptability of the unfermented leaf extracts were most preferred by the panelists and had the highest ratings. In some samples, there was no spoilage of micro-organisms.

Keywords: *Artocarpus communis,* Antinutrients, Fermentation time, Isolates, Leaf extract, Microbial load, Phytochemicals.

INTRODUCTION

Indigenous food crops, edible seeds, and plant products which are widely grown but neglected and rarely consumed by people in urban areas are much more highly nutritious than most exotic foods [1]. *Artocarpus communis* ("ukwa bekee") is one of the seeds which is neglected, underutilized, underdeveloped, and even going into extinct. *A. communis,* a breadfruit belongs to the Mulberry family Moraceae. The name *A. communis* is derived from Greek word, "Artos" bread and "karpus" which refer to the bread-like quality of breadfruit when baked. The fruit is Achene but not a drupe. Other names of *A. communis* which can be used interchangeably are *Artocarpus altilis* and *Artocarpus incisa*. The seeds are edible and are of high nutritional values [2]. As a leguminous crop, *A. communis* is considered as a good source of nutrients such as protein, fats and oils, and a reasonable amount of carbohydrates for both man and livestock feeds for animals [3].

When *A. communis* seeds are cooked, they are fair source of thiamine and vitamin C [4]. In Nigeria, *A. communis* is regarded as the poor man's substitute for yam (*Dioscorea esculenta* and *Dioscorea cayenensis*) due to the fact that it is used in several traditional food preparations of yam and also cost 1/3 the price of yam at the market [5]. The seeds could be cooked for main dish, roasted for snacks or even converted to flour, which can be used for snacks making or as soup thickeners [6]. Fruit (*A. altilis*) is an important food in the pacific [7]. It is widely distributed in the tropics although native to Malaysia, Papua in New Guinea, and the Philippines. Breadfruit (*A. communis*) tree grows easily in a wide range of ecological condition with minimal input of labor or materials and requires a little attention or care [8].

Breadfruits (*A. communis*) are formed from sea level to about 1550 m elation. The latitudinal limits are approximately 17°N and S, but maritime climates extend that range to the tropics of cancer and Capricorn [9]. In Africa, seedless breadfruit (*A. communis*) is found in Senegal, Guinea-Bissau, Cameroon, Sierra Leone, Nigeria, Liberia, and Ghana [10]. According to the Orwa *et al*. [11], *A. communis* is also used as food, fodder, fuel, timber, gum, dye for textiles, and medicine. It is high yielding with an average size tree producing 400-600 fruits per year (NTBG, 2009). Yields are superior to other starchy, staples with a single tree producing between 150 and 200 kg of food [12].

Statement of problem

In general, developing countries do not produce enough foods which have the right nutritional quality to meet daily needs. Therefore, there is a great need to search for more nutritionally balanced food. Furthermore, the projected increases in the human population throughout the developing world and the limited availability of land for increased food and forage production suggest that agricultural production needs to be intensified considerably to satisfy the exalting demand for food [13]. Increased reliance on major food crops has been relied on for generations [14]. Underutilized crops are often presented as new crops [15] for the fact that commercial companies/researchers

are only recently giving attention to them. In reality, local populations over generations have used these species. The loss of local knowledge and the increasing ignorance of new generations on the traditional uses of these crops also contribute to portraying such a misleading image [13].

Researches carried out show an indication that breadfruit leaf extracts contain chemicals that are similar to antimicrobial medicine. These are chemically diverse compounds as secondary metabolites (phytochemicals) which include such well-known substances such as glycosides, terpenes, sterols, tannins, flavonoid, phenols, and resins among others [16]. The investigation from other researchers provides supportive data for the use of *A. communis* as well as some of its constituents for the treatment of infections affected with the studied micro-organisms. This would be confirmed with further pharmacological (*in vivo* activity) bioavailability and toxicological studies using animal models [17]. Consequently, these extracts could be used as skin lightening agents for treating hyperpigmentation disorder and might be used as active ingredient in skin care products for preventing the darkening of the skin. However, their safety and efficacy in human would need to be examined in future studies as reported by Hsu and Chang [18]. It is necessary to create awareness of nutritional and medicinal benefits of breadfruit which would help to improve consumption levels. Furthermore, the increasing awareness about its nutritional qualities is a major challenge in question to transform breadfruit from its hidden identity into a crop that enhances livelihood.

Breadfruits (*A. communis*), generally, are not in Nigeria as a food crop. They usually grow wild and are left unprotected. As a result, important accessions might be lost. Breadfruits (*A. communis*) are covered by the international treaty on plant genetics and are considered threatened. Information is limited on the nutrient, antinutrient, and phytochemical composition of processed and unprocessed *A. communis* leaves in Nigeria. The aim of this work was to determine the effect of fermentation time (0, 3, 6, 9, and 12 hrs) on the nutritional, phytochemical, and microbiological quality of seedless breadfruit (*A. communis*) leaf extracts as well as to evaluate the sensory and microbiological properties of the formulated leaf extract of *A. communis* at different period of fermentation); and then, to isolate and biochemically characterize the micro-organisms.

METHODS

Procurement of materials

A. communis leaves were harvested from a garden at Iheakpu-Akwa village in Nsukka Local Government area of Enugu State, Nigeria.

Preparation of sample

A. communis leaves were freshly harvested by manual plucking. After plucking and sorting, they were subjected to washing, size reduction (cutting with knife) weighing, and fermentation, blending/milling, sieving with muslin cloth, extraction and heat treatment (pasteurization) as shown in Fig. 1. Fermentation was at room temperature at different intervals (0, 3, 6, 9, and 12 hrs). 300 g of the samples was weighed after cutting (2-4 mm) was soaked in a bucket containing 500 ml of portable water. Pasteurization was at 77°C for 1 minute. The 0-hr sample (which serves as a control) was wet milled using a blender (Binatone blender model No BLG-450) without undergoing fermentation process and it was sieved to get the leaf extract and pasteurized, filled and then bottled. Then, 500 ml of portable water was used to blend 300 g of each sample at different periods of fermentation. The leaf extract obtained from 0-hr (control) regarded as raw and leaf extract obtained from different periods of fermentation (processed) were used for the analysis.

Proximate analysis

Determination of moisture content

The moisture content of the sample was determined according to the standard procedure of AOAC (2010) [19]. The crucibles were washed and dried in an oven at 100°C for 1 hr. The weight was noted as w_1. Then, 2 ml of each sample was separately weighed into the crucibles and their weights taken as (w_2) before and during drying at 100°C to constant weight (w_3).

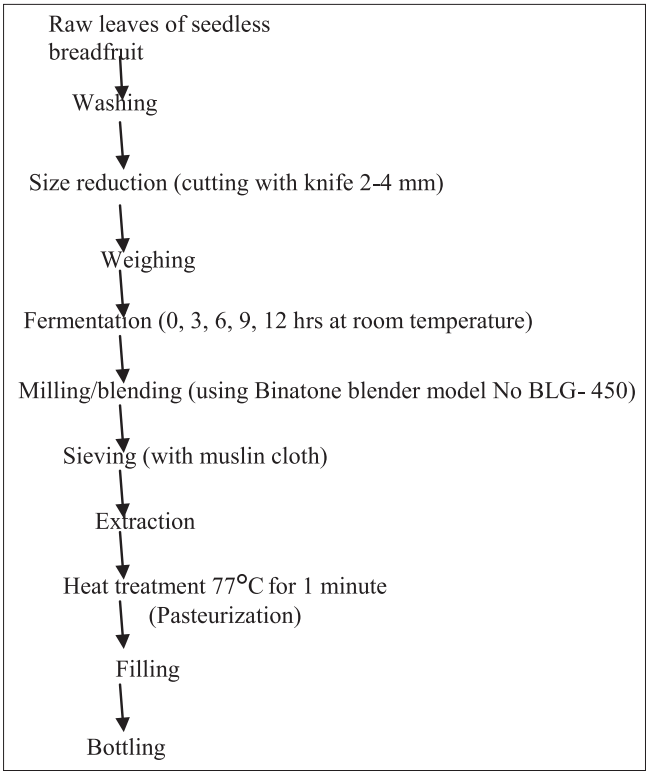

Fig. 1: Flow diagram of processing of *Artocarpus communis* leaves into leaf extract

$$\% \text{ moisture} = \frac{\text{Weight of moisture}}{\text{Weight of sample}} \times 100 = \frac{w_2 - w_3}{w_1} \times 100$$

Where,
w_1=Weight of empty crucible;
w_2=Weight of crucible and sample before drying;
w_3=Weight of crucible+Weight of sample after drying to a constant weight.

Determination of protein content

The protein content of the samples was determined according to the standard methods of AOAC (2010) [19] using Kjeldahl's method.

Digestion of the sample

2 ml of a sample was weighed into Kjeldahl's flask and anhydrous sodium sulfate of about 5 g was added. 25 ml of concentration H_2SO_4 was added with few boiling chips. The content of the flask was heated in the fume chamber until clear solution was obtained. The solution was cooled and transferred into 250 ml volumetric flask and made up to the level with distilled water.

Distillation

The distillation unit was carried out using a well cleaned Markham apparatus 100 ml conical flask (receiving flask) containing 5 ml of 2% boric and 2 drops of methyl red indicator was placed under the condenser. Then, 5 ml of the digest was pipetted into the apparatus through the small funnel on the distillation unit. The digest was washed down with distilled water. Followed by addition of 10 ml of 60% sodium hydroxide.

Titration
The solution in the flask was then titrated with 0.01 N HC1 until the first permanent pink color appears. The blank was titrated in the same way:

$$\% \text{ Nitrogen} = \frac{V_s - V_b \times N\text{acid}}{w} \times 100$$

Where,
V_s=Volume (ml) of acid required to titrate sample;
V_b=Volume (ml) of acid required to titrate the blank;
N (acid=Normality of acid (0.1 N); w=Weight of sample in gram (g);
Therefore, protein (%)=N×6.25 (conversion factor for protein).

Determination of fat content

The fat content of the samples was determined using the standard AOAC (2010) [19] method. A Soxhlet extractor was a reflux condenser, and a 500 ml round bottom flask was set up. Then, 300 ml of petroleum ether was paired into the round bottom flask. The sample (2 ml) was weighed into labeled thimble and sealed with cotton wool, then fitted into the extraction tube of the Soxhlet extractor. The Soxhlet extractor after assembly was allowed to reflux for about 6 hrs, after which the thimble was removed with care and the petroleum ether (40-60°C) collected on top and drained into a container for re-use. The flask and its content was dried between 50°C and 60°C in a hot air oven. It was then removed from the oven and cooled in a desiccator and weighed.

$$\text{Fat } (\%) \text{ content} = \frac{w_2 - w_1}{w} \times 100$$

Where,
w=Weight of sample used;
w_1= Weight of empty extracting flask;
w_2=Weight of flask and extracted oil.

Determination of ash content

The ash content of the samples was determined according to the standard method of AOAC (2010) [19]. A preheated and cooled crucible was weighed (w_1). Approximately, 2 ml of the sample was weighed into the crucible (w_2). The crucible was sent to the hot air oven to dry up the liquid content so as to avoid spurting. The sample was charred on a Bunsen flame inside a fume cupboard. The charred sample was placed in a muffle furnace set at 550°C for 2 hrs until a white or light grey ash was obtained (w_3). The sample was removed, cooled in desiccators, and weighed.

$$\text{Fat } (\%) \text{ content} = \frac{w_2 - w_1}{w} \times 100$$

Where,
w=Weight of sample used;
w_1=Weight of empty extracting flask;
w_2=Weight of flask and extracted oil.

Determination of carbohydrate content

The carbohydrate content was determined by difference as illustrated by AOAC (2010) [19]. The sum of all other proximate analysis tests (% moisture, % ash, % protein, and % fat) were calculated and the total was subtracted from 100% carbohydrate=100−(% moisture+% ash+% protein+% fat).

Determination of antinutrients

Determination of phytic acid
The method described by Oberleas [20] was used for the determination of phytic acid. 2 ml of the sample was weighed into a 100 ml flask and extracted with 50 ml of 0.2N HC1. 5 ml of the extract was measured out into a test tube fitted with a glass stopper. 1 ml of solution prepared by dissolving 0.2 g ammonium iron (III) sulfate $12H_2O$ in 2 N HC1 and made up to 100 ml with distilled water was added to the extract. The tube was heated in a boiling water bath for 30 minutes and cooled in the water for 15 minutes before allowing it to adjust to room temperature. The contents of the tube were mixed and centrifuged for 30 minutes at 3000 rpm. 1 ml of the supernatant was transferred to another test tube, and 1.5 ml of solution made by dissolving 10 g 2, 2-bipyridine and 100 ml thioglycolic acid in distilled water and made up to 1000 ml was added to it. The absorbance was measured at 519 nm against distilled water. Calibration curve was prepared by plotting the concentration of the reference solution (phytate reference solution) against their corresponding absorbance. The absorbance of the test tube sample was then used to obtain the concentration from the calibration curve.

Determination of alkaloid
Alkaloid was determined according to Habourne [21]. About 20 ml of the samples was soaked in a solution of 10% acetic acid in ethanol and allowed to stand for 4 hrs. The mixture was filtered, and the filtrate was concentrated to one-quarter of its original volume over a steam bath. Moreover, concentrated NH_4OH was added dropwise until a precipitate occurred. The crude alkaloid was collected by centrifugation.

$$\text{mg alkaloid}/100 \text{ g sample} = \frac{\text{Weight of residue}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of saponin
Saponin content was determined by the procedure described by AOAC (2010) [19]. 2 ml of the sample was folded into a thimble and put in a Soxhlet extractor and a reflux condenser fitted on top. An extraction will be done with 200 ml of acetone in a 250 cm^3 capacity round bottom flask for 3 hrs. The weight of the flask was taken before and after the extraction to know the change in weight. The methanol was used in the same way for the second extraction. At the end, it was oven-dried to remove any remaining solvent in the flask. The flask was cooled in desiccators and weighed. The percentage of saponin was calculated as follows:

$$\% \text{ Saponin} = \frac{\text{Weight of dry extract}}{\text{Weight of sample}} \times \frac{100}{1}$$

Determination of oxalate
The titration method (AOAC, 2010) [19] was used for the determination of oxalic acid in the sample. The method involves three major stages: Digestion, oxalate precipitation, and $KMnO_4$ titration.

Digestion
The dried and ground sample (2 ml) was suspended in 190 ml of distilled water in a 250 ml volumetric flask. 10 ml of 6 M HCI was added and the suspension heated on a water bath at 100°C for 1 hr. The mixture was cooled and made up to 250 ml mark with distilled water before filtration.

Oxalate precipitation
A duplicate portion of 125 ml of the filtrate was measured into beakers. Each extract was made alkaline with concentrated NaOH. Each portion was heated to 90°C, cooled and filtered to remove precipitate containing ferrous ions. The filtrate was heated again to 90°C on a water bath, and 10 ml of 5% calcium chloride solution added while being stirred constantly. After heating, it was cooled and left overnight at 5°C. The solution was centrifuged at 2500 rpm and the supernatant decanted. The precipitate was completely dissolved in 10 ml of 20% (v/v) tetraoxosulphate (VI) solution, and total filtrate resulting from 2 ml of the sample was made up to 300 ml.

Permanganate filtration
Aliquots of 125 ml of the filtrate were heated until near boiling and the titrated against 0.05 ml potassium permanganate solution until a pink

color persists for 30 seconds. The oxalate content was calculated using the formula:

$$\% \text{ oxalic acid} = \frac{K}{\text{sample size}} \times \frac{100}{1}$$

$$\Rightarrow K = \frac{2.7 \times 10 \times 0.0025}{5}$$

$$K = \left[\frac{\text{Mean titre} \times DF \times \text{oxalic factor}}{\text{Molar equivalent of } KMnO_4} \right]$$

Where,
DF=Dilution factor

Determination of phytochemicals

Determination of carotenoid

The determination of total carotenoid was done using AOAC (2010) [19] procedure. 5 ml of the sample was crushed in a mortar and blended with hexane and ethanol at a ratio of 40 ml hexane to 60 ml ethanol in a warring blender (Binatone blender, BLG-450, China) to extract the carotenoid. The mixture was transferred to a separating funnel and swirlled vigorously after adding 2% NaC1 solution. It was allowed to settle and the lower layer ran off. The top layer, which contains the carotenoid, was collected. It was diluted and optical density determined in a spectrophotometer (115 V, AC, 60 H_Z, Cole-palmer UV/visible spectrophotometer, England) at 460 nm. The result was compared with carotenoid standard curve to be prepared using carotenoid stock solution starting from 0.1 to 0.8 mg/ml of hexane. The content of carotenoid in the sample was calculated using the formula:

$$\text{mg carotenoid}/100\text{g sample} = \text{concentration} \times \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$$

Determination of flavonoid

The flavonoid was determined using the method of Habourne [21]. 5 ml was boiled in 50 ml of 2 M HCl solution for 30 minutes under reflux. It was allowed to cool and then filtered through Whatman No. 42 filter paper. A measured volume of the extract (20 ml) was treated with equal volume of ethyl acetate starting will drop. The flavonoid precipitated was recovered by filtration using weighed filter paper. The resulting weight difference was taken as the weight of flavonoid in the sample.

Determination of tannin

This was determined by Van-Buren and Robinson (1981) method. Then, 0.5 ml of the sample was weighed into 150 ml bottle. Then, 50 ml of distilled water was added and shaken for 1 hr in a mechanical shaker. This was filtered into 150 ml volumetric flask and was made up to the mark. The filtrate (5 ml) was pipetted out into a test tube and mixed with 3 ml of 0.1 M $FeC1_3$ in 0.1 N HC1 and 0.008 M potassium ferrocyanide. The absorbance was measured at spectrophotometer (115 VAC, 60 H_Z, Cole-palmer UV/visible spectrophotometer, England) at 720 nm wavelength within 10 minutes. A blank sample was prepared and the color developed and read at the same wavelength. A standard was prepared using tannin acid to get 100 ppm and was measured as shown below:

$$\text{Tannin } (\text{mg}/100\text{ ml}) = \frac{\text{Concentration of standard}}{\text{Concentration of sample}} \times \frac{\text{Absorbance of sample}}{\text{Absorbance of standard}}$$

Determination of phenol

Phenol content was done using Obadoni and Ochuko [22] method. 2 ml of the sample was initially defatted for 2 hrs with 100 ml of petroleum ether using the Soxhlet apparatus. The fat-free sample was boiled with 50 ml of ether for the extraction of the phenolic component for 15 minutes. The extract (5 ml) was pipette into 50 ml volumetric flask and 10 ml of distilled water added. A pinch of potassium ferricyanide (2 ml) of NH_4OH solution and 5 ml of concentrated amyl alcohol was added. The sample was made up to 50 ml mark and allowed to stand for 30 minutes for color development. The absorbance was measured at 505 nm wavelength, using a spectrophotometer (Genway spectrophotometer 6305, England). A standard solution was prepared at 0.0, 2.00, 4.00, 6.00, 8.00, and 10.00 ppm with the same treatment. The concentration of the sample (in ppm) measures from the curve was represented as *x* 5 ml of the extract contained:

$$\frac{50 \text{ ml} \times 5 \times \mu\text{g phenol}}{5}$$

$$\text{Since 2 g sample was used, therefore, phenol } (\%) = \frac{50 \times 50x}{5} \times \frac{1}{10^6} \times \frac{100}{2} = 0.025x\%$$

Determination of vitamin content

Determination of Vitamin C (Ascorbic acid)

Ascorbic acid content was determined using AOAC (2010) [19] method. 2 ml sample was homogenized with acetic acid solution and extracted. Vitamin C standard solution was prepared by dissolving 50 mg standard ascorbic acid tablet in 100 ml volumetric flask with distilled water. The solution was filtered out and 10 ml of the clear filtrate added into a conical flask in which 2.5 ml acetone has been added. This was titrated with indophenol dye solution (2, 6-dichlorophenol indophenol) for 15 seconds.

$$(\text{mg}/100\text{ ml}) = \frac{V \times \frac{W}{Y} \times Df}{\text{Weight of Sample}} \Rightarrow 20 = \text{Vitamin C in mg}/100\text{g}$$

Where,
V=Titer of sample solution;
W=Weight of vitamin C standard;
Df=Dilution factor;
Y=Titer value of standard vitamin C solution.

Determination of vitamin E (tocopherol)

Vitamin E contents were determined by the procedure as described by Pearson [23]. l ml of the sample was extracted with some of petroleum ether and concentrated to dryness. The residue potassium hydroxide under reflux. Then, 20 ml of petroleum ether was used to extract the unsaponifiable matter and concentrated to dryness. Furthermore, 20 ml ethanol was added to dissolve the residue, 1 ml was transferred to 3 test tubes, and 1 ml 0.2% ferric chloride in the ethanol was added and 1 ml of 0.5% indicator dye in ethanol was made up to 5 ml with ethanol. The absorbance was taken at 520 nm against a blank.

Determination of vitamin B1 (Thiamin)

Thiamine content was determined by AOAC (2010) [19] method. A 2 ml of the sample was weighed into a conical flask, 75 ml of 0.2 N HCl was added and the mixture incubated at 37 overnight. The solution was filtered and the filtrate purified by passing through silicate column. To 25 ml of the filtrate in a conical flask was added 5 ml acidic KC1 eluate, 3 ml of alkaline ferricyanide solution, and 15 ml isobutanol, and shaken for 3 minutes. After the separation of the solution, the alcohol layer was taken. Anhydrous sodium sulfate (3 g) was added to the alcohol layer. To prepare the standard 5 ml of thiamine solution, a 3 ml of 15% NaOH was be added to the flask instead of alkaline ferricyanide. The blank sample was poured into fluorescence reading tube and reading taken at the expression;

$$\text{Thiamine } (\%) = \frac{X}{Y} \times \frac{1}{5} \times \frac{25}{V} \times \frac{100}{W}$$

Where,
W=Weight of sample;
X=Reading of sample−Reading of blank sample;
Y=Reading of thiamine standard−Reading of blank sample;
V=Volume of solution used for test on the column.

Determination of riboflavin (vitamin B_2) content
Riboflavin was determined using AOAC (2010) [19] procedure. The sample (2 ml) was washed into a conical flask and 50 ml of 0.2 N HCI added. The mixture was boiled for 1 hr, and allowed to cool. Sodium hydroxide was added to adjust the pH of the solution to 6.0. Then, 10 ml HCI was added to the sample solution to lower the pH to 4.5. The solution was then filtered into 100 ml volumetric the flask and made-up to and labeled 1 and 2. About 10 ml of water was added to tube 1. Another 10 ml of filtrate and 1 ml of riboflavin standard was added to test tube 2. Moreover, 1 ml of glacial acetic acid was added to each tube and mixed. Then, 0.5 ml 3% $KMnO_4$ solution was added to each tube. The test tube was allowed to stand for 2 minutes, after which 0.5 ml 5% H_2SO_4 was then be added and the solution mixed well. The flourimeter was adjusted to excitation wavelength of 525 nm. The flourimeter was also adjusted to zero deflection against 0.1 N H_2SO_4 and 100 against tube 2 (standard). The fluorescence of tube (l) was added to both tube and the fluorescence measured within 10 seconds. Riboflavin was then calculated as follows:

$$\text{Riboflavin } (\text{mg/g}) = \frac{\text{Absorbance of standard}}{\text{Absorbance of sample}} \times \frac{\text{Concentration of sample}}{\text{Concentration of standard}}$$

Determination of mineral content

Determination of magnesium content
Magnesium content was determined by the procedure as described by AOAC (2010) [19]. Then, 75 ml of the sample was measured into a beaker. 2 ml of buffer solution and 1 ml of 5% ammonium oxalate solution was added. The mixture was allowed to stand for 5-10 minutes then filter through Whatman No 1 filter paper and the first 5-10 ml of the filtrate was discarded. Furthermore, 25 ml of the filtered sample was diluted to 50 ml with distilled water and 2 drops of indicator solution was added. Standard EDTA titrant was added slowly, with continuous stirring until the last reddish tinge disappears from the solution. At the end point, the solution obtained was normally blue in color. However, a distilled water blank of the same volume as the sample was titrated and identical amounts of buffer, inhibitor, and indicator solution was added. Magnesium content was calculated.

Magnesuim (mg/100 ml)=1 ml/sample size×df/1

Where,
Df=Dilution factor

Determination of calcium
Calcium content was determined using the method of AOAC (2010) [19]. 1 ml of the sample was digested with 20 ml of acid mixture (650 ml concentrated HNO_3, 20 ml concentrated H_2SO_4). The mixture was heated until a clear digest was obtained. The digest was made up to 500 ml with distilled water. The determination was done using complexometric (EDTA) filtrations; calcium indicator was used as a masking agent. An aliquot of the extract was added in 250 cm^3 conical flask and diluted with 50 cm^3 of de-ionized water. A pinch of calcium indicator mixture (Calcium - K_2SO_4) was added and titrated with M/50 EDTA solution to a non-fluorescent orange end point. A blank determination was done and calcium content was calculated.

Calcium (mg/100 ml)=1 ml/sample size×df/1

Where,
df=Dilution factor

Determination of iron content
The iron content was determined using dry weight basin as described by AOAC (2010) [19]. A sample (0.5 g) of the oven-dried sample was added into 20 ml of 1.1 mixtures of concentrated nitric and perchloric acid. The digestion was done in a fume cupboard using hot plate until the white fume appears which shows that digestion is complete. The solution was cooled and filtered. 10 ml aliquot of the ash solution was added in a volumetric flask. 2 ml of 10% hydroxylamine was added into the flask and mixed thoroughly. Then, 20 ml of 0.20% of the orthophenanthroline solution was added and the volume was made up to 100 ml with distilled water. The flask was shaken vigorously and was allowed to stand for 20 minutes for the color to develop. After 20 minutes, the absorbance at 510 nm was read using a spectrophotometer (Spectronic 20 spectrophotometer 6302, England).

Determination of phosphorus content
The phosphorus content of the sample was determined by the spectrophotometer vanado Molybdenum method as describe by AOAC (2010) [19]. Around 5 ml of the sample from the wet digested extract was measured into a 50 ml volumetric flask, followed by addition of 10 ml of the color developing agent are made up to 50 ml with distilled water. The mixture was allowed to stand for 10 minutes to allow for color development. The sample from the mixture was run in a spectrophotometer at 400 nm wavelength and readings from 0 to 5 ppm were prepared from a standard solution of phosphorus. The absorbance was read against a blank and number of milligrams of phosphorus equivalent was calculated:

$$\text{Concentration of phosphorus} = \frac{\text{Absorbance of test sample}}{\text{Absorbance of standard}} \times \frac{\text{Concentration of sample}}{\text{weight of sample used}}$$

Microbiological analysis

Determination of total viable count

The total viable count was determined by the method described by Harrigan and McCance [24]. The media (saline solution) was prepared by 10-fold serial dilution. 1 ml of the diluted sample extract from the second tube was taken and transferred into a third tube containing 9 ml of buffer peptone water and was shaken to get the third dilution. 1 ml of the dilute sample extract from the third tube was taken and transferred into a fourth tube containing 9 ml of buffer peptone water and was shaken to get the fourth dilution. 1 ml of the diluted extract from the fourth tube was taken and transferred into a fifth tube containing 9 ml of buffer peptone water and was shaken to get the fifth dilution (Serial dilution). From the 10^{-5} dilution, 1 ml of the suspension was inoculated with nutrient agar using pour plate method and incubated for 24 hrs at 37°C. The number of colonies was counted using colony counter.

The total viable count was calculated using the formula below:

$$\text{TVC} = \frac{\text{Mean count/drop} \times \text{reciprocal of dilution factor}}{\text{volume/drop}}$$

Where,
Reciprocal dilution factor= $1/10^5$;
Estimated volume per drop=0.015 ml.

Cultivation of microorganism

The cultivation was done using the procedure as described by Harrigan and McCance [24]. The plant materials previously macerated and bottles were used. This involved the growing of the suspected organism from the sample bottles into a newly prepared medium preferably a general purpose medium (Nutrient agar). This medium was prepared poured in sterile plates, allowed to set, and was allowed to cool before cultivation by streaking and was followed by incubation at 37°C for 24 hrs.

Isolation of micro-organism

The nutrient agar was sterilized at 121°C and 15 lb pressure for 15 minutes in an autoclave. This was poured in the nutrient agar glass plates (sterilized in hot air oven at 160°C for 1 hr) and was dried at 60°C for 30 minutes. A sample from dilution of 10^{-3} or 10^{-5} preparation was individually made on the agar and streaked using an inoculating hoop which was the first flamed over the Bunsen burner. This was incubated at 37°C for 24 hrs. The isolated was characterized presumptively by colonial morphology, spore production, gram reaction, and

biochemical tests. The isolates were thereafter subcultured on agar slants and incubated for 24 hrs at 37°C. Appropriate morphological and biochemical tests were carried out to further characterize the organisms [24].

Colonial morphology

After isolation and purification of isolates, the growth on MacConkey agar was observed for colonial morphology which includes the shape, size, chromogenesis, opacity, elevation, surface, edge, consistency, emulsifiability, and odor of the isolates [24].

Microscopy

After colonial morphology organisms was observed for gram reaction, spore staining, and capsule staining.

Gram's staining

Gram stain was done using the method described by Harrigan and McCance [24]. A heat-fixed smear was prepared from an 18-24 hrs culture from nutrient agar broth, was stained with crystal violet for 1-2 minutes, and then, was rinsed with water and Gram's iodine solution was added and left for 1 minute. The iodine was poured off and blotted dry. The slide was washed with 95% ethanol (or industrial methylated spirit) until no more stain run from the slide (only 5-15 seconds in the case of well-prepared smears). The slide was rinsed under the tap and stained with dilute carbol fuchsin solution for 20 seconds. The slide was washed well and blotted dry. The slide was viewed under a microscope using oil immersion.

Spore staining

Spore staining was done using method described by Harrigan and McCance [24]. The organism was smeared and heat-fixed to a slide. The slide was placed over a steam bath covered with Malachite green. The stain was left over the steam bath for 3-5 minutes recovering the slides with Malachite green if some evaporated. The Malachite green was dumped and allow to cool. The slide was rinsed with water to remove excess stain, and the smear was covered with Safranin for 2 minutes. The stain was blotted and viewed under oil immersion microscope.

Capsule staining

A few drops of Congo red (aqueous solution) was placed on a clean slide and mixed in a small amount of culture. The slide was placed on a rack of the staining tray and the smear was gently flood with crystal violet, allowed to stand for 5 minutes and water was allowed to rinse the slide. The slide was removed from tray and placed on absorbent paper. The sample was allowed to dry and the slide was viewed under a microscopy using an oil immersion microscope [24].

Biochemical characterization of micro-organisms

In general, other tests in addition to the staining reactions are important in the identification of bacteria. Some bacterial species have similar morphological, cultural or ever staining reaction, which make exhaustive biochemical and other tests important in bacterial identification. These biochemical tests include sugar fermentation test citrate test, catalase test among others according Harrigan and McCance [24].

Sugar fermentation test

This was done using composed peptone water sugar. The indicator solution for the basal medium was prepared by dissolving 0.1 g of bromothymol blue in 2.5 ml of 0.1 mole per liter of NaOH, 47.5 ml of sterile distilled water was added and mixed well. About 1000 ml of basal medium was therefore gotten by the addition of 100 g of peptone, 12.5 ml of indicator, and 5 g sodium chloride for the preparation. The pH was adjusted to 7.4. Then, 200 ml amount of the mixture was dispensed into stoppered conical flasks and autoclaved at 121°C and 15 lb pressure for 15 minutes. Then, 0.5% peptone water sugar was also compounded by the dissolution of 5 g of the different sugars in 50 ml distilled water and dispensed into Bijou Bottles in 10 ml ampoules [24].

Citrate test

The Simmons' Citrate medium was used. A 23 g of the dehydrated medium was suspended in a 1 L of distilled water and homogenized 8 ml of the medium was dispensed into test tubes plugged with cotton wool and kept in slants. Pure isolates were inoculated into the sterile medium and incubated at 37°C for 24-48 hrs. Growth and blue coloration shows positive test because of the change in indicator color from green to blue was indicated the use of citrate as sole carbon source. No change in color of medium (green) and no growth was indicated a negative test [24].

Catalase test

Catalase test was done using the method described by Harrigan and McCance [24]. About 2-3 ml of the hydrogen peroxide (H_2O_2) solution was poured into a test tube. Using a sterile wooden stick or glass rod. A good growth (a loopful) of the test organism was removed and immersed it in the H_2O_2 solution. The presence or absence of immediate bubbling (effervescence) caused by the liberation of free oxygen as gas bubbles indicated the presence of catalase in the culture under test) was looked for. Active bubbling (positive test) was shown that catalase was produced as shown in the equation below while no release of bubbles was indicated negative test and no catalase was produced.

$$2H_2O_2 \longrightarrow 2H_2O + O_2$$

Hydrogen peroxide Water Oxygen

Sensory evaluation

The formulated leaf extract obtained from 0, 3, 6, 9 and 12 hrs fermentation were used for sensory evaluation. The samples were evaluated using a 30-member untrained panelist consisting of students from Department of Food Science and Technology, University of Nigeria, Nsukka according to Ihekoronye and Ngoddy [25]. Quality attributes such as color, flavor, taste, aftertaste, aroma, appearance, and overall acceptability were evaluated using nine-point Hedonic scale (when 1 represents dislike extremely and 9 represents like extremely).

Data analysis/Experimental design

Means were analyzed using one-way analysis of variance based on completely randomized design. Mean separation was by Duncan's New multiple range test [26].

RESULTS AND DISCUSSION

Proximate composition of fermented and unfermented seedless breadfruit (*A. communis)* leaf extracts

The proximate composition (%) of fermented and unfermented seedless breadfruit *(A. communis)* leaf extracts are shown in Table 1.

The moisture content of the extract prepared from the unfermented sample was 96.62% while the moisture content of the fermented extracts ranged from 96.70% to 98.55%. There was a significant increase in the moisture content of the extract. This was probably due to the fact that the products were all formulated with water. The moisture content of any food is an index of its water activity [27] and is used as a measure of stability and susceptibility to microbial contamination [28]. This implied that the extracts might have a short shelf life due to its high moisture. Hence, adequate preservative measures are recommended to reduce the immediate spoilage of the product. This informed the adequate processing measures taken (bottling and pasteurization) in the preparation of the extracts.

The protein content of the unfermented breadfruit (*A. communis*) leaves extract was 2.48% while the protein content decreased to a range of 0.98-2.30%. Kuti and Torres [29] had already reported

that fermentation decreased the relative composition of the protein. Moreover, the drastic decrease in protein content of the leaves extracts could be as a result of high moisture contents in the extract resulting from the addition of water during formulation. Ihekoronye and Ngoddy [25] stated that protein in vegetables and their products are low but have high quality.

The fat content of breadfruit leaves extract ranged from 0.3% to 0.56% where unfermented sample (0.56%) was the highest and fermented sample for 9 hrs was the least. In general, it was observed that fat content was lower in fermented samples than unfermented sample. This could probably be due to utilization of fat by micro-organisms during fermentation as reported by Adebowale and Maliki [30]. Although the fat is low but is still higher than fat content of *Amaranthus hybridus* leaf (0.2 g) according to Oguntona [31].

The ash content ranged from 0.16% to 0.35% where the breadfruit (*A. communis*) leaves extract fermented for 6 hrs was the highest and fermented leaves extract for 9 hrs was the lowest. It was observed that there was decrease in the ash content during fermentation except fermented extract in water for 6 hrs. This might have resulted from some of the inorganic salts which were affected during fermentation of vegetable [32].

The carbohydrate content of the breadfruit (*A. communis*) leave extract prepared from the unfermented sample was 0.05%, while the carbohydrate content of the extract prepared from the fermented samples ranged from 0.00% to 0.19%. In general, it was observed that carbohydrate content was low. A steady decline in carbohydrate content of some Nigerian leafy vegetables such as *Telfairia occidentalis* (fluted pumpkin) due to respiratory loss of sugars as CO_2 had been reported by (Okoli *et al*., 1988). Dirar [32] also observed that fermentation decline the carbohydrate content of some Nigerian leafy vegetable such as *T. occidentalis* and *Vernonia amygdalina.*

Antinutrient composition of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts

The results of the antinutrient content of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts are shown in Table 2.

The phytate content of the extract prepared from the unfermented leaves was 6.80 mg/100 ml, while the phytate content decreased to a range of 0.81-3.39 mg/100 ml for fermented extracts. Marfo *et al.* [33] reported that the level of phytate determined in *Euphorbia hirta* raw leaves was 6.67 mg/100 ml which fall within the same range of *A. communis* leaves unfermented 0-h, that is, raw leaves. Marfo *et al.* [33] also reported that low level of phytate in leaves would be nutritionally advantageous.

The alkaloid content of the unfermented leaves extract was 0.09% while that of fermented leaves extract ranged from 0.16% to 0.38%. It was observed that the alkaloid content increased with fermentation at the different intervals but was lower than the range of 0.82-1.81 mg/100 g reported for some fresh Nigerian leafy vegetables such as "utazi," "uturukpa," "ugu," and "okazi" [34].

Oxalate content of the fermented leaves extract ranged from 2.07 to 3.17 mg/100 ml. The unfermented leaves extract had the highest oxalate value of 4.90 mg/100 ml. The reduction in oxalate content could probably be attributed to the fact that oxalates in the vegetables are removed when vegetables were washed and fermented [35].

The saponin content of the extract prepared from the unfermented leaves was 0.18% while the values for the fermented extract ranged from 0.07% to 0.14%. The decrease in the saponin content could probably be attributed to the activities of micro-organisms during fermentation at different time interval [36].

Phytochemical composition of fermented and unfermented seedless breadfruit (*A. communis*) leaf extracts

The phytochemical composition of fermented and unfermented seedless breadfruit (*A. communis*) leaf extracts are shown in Table 3.

The result of the carotenoid content of the seedless breadfruit (*A. communis*) leaves extract showed variations. The values for the fermented extract ranges from 0.07% to 0.90% while the extract from the unfermented was 0.04%. This showed that there was decrease with fermentation, which was in agreement with the findings of Steinkraus [37] that fermentation increased the carotenoid of fermented *A. hybridus* leaves.

The tannin content of the extract prepared from the unfermented leaves was 20.83 mg/100 ml while that of fermented leaves extract ranged from (16.13 to 30.04 mg/100 ml). This showed that tannin content increased with fermentation time except for 12 hrs (16.13 mg/100 ml), which was the least. The values were lower than the tannin content of fermented *V. amygdalina* (bitterleaf) which ranged from 207.41 to 286.42 mg/100 ml as reported by Agbaire [38].

Phenol content of the fermented leaves extract ranged from 0.08 to 0.26 mg/ml. Unfermented leaves extract had the highest phenol value (0.48 mg/ml). This showed that *A. communis* leaves had phenolic acid found in leaves and seeds of the most plant and had strong antioxidant properties that make it especially useful in the formation of anti-aging compound [39].

Table 1: Proximate composition of fermented and unfermented seedless breadfruit (*Artocarpus communis*) leaf extracts

Samples	Time (h)	Moisture	Protein	Fat	Ash	Carbohydrate
Unfermented	0	96.62^{a}±0.25	2.48^{d}±0.17	0.56^{d}±0.03	0.29^{b}±0.02	0.05^{ab}±0.04
Fermented	3	98.03^{c}±0.13	1.41^{b}±0.11	0.38^{b}±0.00	0.18^{a}±0.02	0.00^{a}±0.00
Fermented	6	97.35^{d}±0.26	1.83^{c}±0.19	0.40^{b}±0.01	0.35^{c}±0.01	0.06^{b}±0.06
Fermented	9	98.55^{d}±0.22	0.98^{a}±0.20	0.30^{a}±0.02	0.16^{a}±0.02	0.00^{a}±0.00
Fermented	12	96.70^{a}±0.02	2.30^{d}±0.01	0.50^{c}±0.01	0.30^{b}±0.01	0.19^{c}±0.01

Values are mean±standard deviation of triplicate determinations. Values bearing different superscripts within the same column are significantly different ($p<0.05$)

Table 2: Antinutrient composition of unfermented and fermented seedless breadfruit (*Artocarpus communis*) leaf extracts

Samples	Time (h)	Phytate (mg/100 ml)	Alkaloid (%)	Oxalate (mg/100 ml)	Saponin (%)
Unfermented	0	6.80^{e}±0.21	0.09^{b}±0.00	4.10^{d}±0.20	0.18^{c}±0.02
Fermented	3	3.39^{d}±0.11	0.16^{c}±0.04	3.17^{a}±0.12	0.10^{a}±0.01
Fermented	6	2.82^{c}±0.27	0.22^{d}±0.02	2.60^{b}±0.00	0.07^{a}±0.03
Fermented	9	1.16^{b}±0.09	0.29^{e}±0.04	2.37^{ab}±0.25	0.09^{a}±0.00
Fermented	12	0.81^{a}±0.12	0.38^{a}±0.01	2.07^{a}±0.31	0.14^{b}±0.04

Values are mean±standard deviation of triplicate determinations. Values bearing different superscripts within the same column are significantly different ($p<0.05$)

The flavonoid content of the unfermented leaves extract was 1.28%. The fermented leaves extract ranged from 0.66% to 0.84%. It was observed that unfermented leaves extract had the highest value. This could probably be due to high flavonoid content which indicates high antioxidant potential that could be of beneficial effect to health [40].

Vitamin composition of fermented and unfermented seedless, breadfruit (*A. communis*) leaf extracts

The vitamin composition (mg/100 ml) of fermented and unfermented seedless, breadfruit (*A. communis*) leaf extracts are shown in Table 4.

The vitamin C content of the extract prepared from the unfermented sample was 26.49 mg/100 ml while that of fermented samples ranged from 15.54 to 18.51 mg/100 ml. The reduction could probably be attributed to oxidation. Vitamin C is highly soluble. The value of the vitamin C obtained for the fresh sample unfermented is lower than those of other commonly consumed green leafy vegetables in Nigeria. According to Ogunlesi *et al*. [41], "uziza' leaves (*Piper guineense*) had 59.01 mg 100 g while bitterleaf *(V. amygdalina*) had 125.17 mg/100 g. "Oka" leaves (*Pterocarpus mildbraedii)* had 129.00 mg/100 g and "utazi" leaves (*Gongronema latifolium*) contained 100.00 mg/100 g.

Vitamin E content of the fermented leaves extract ranged from 0.02 to 0.06 mg/100 ml. Unfermented leaves extract was 0.01 mg/100 ml. It was observed that the vitamin E value of the fermented samples was higher than that of the unfermented. It had been reported that fermentation processes could result in increased levels of microbial metabolites in the vegetables [42].

The vitamin B_1 content of the unfermented leaves extract was 0.46 mg/100 ml while that of the fermented ranged from 0.46 to 0.49 mg/100 ml. The vitamin B_1 value for unfermented sample was higher than those reported for green beans (0.13 mg/100 g) but lower than that of spinach (0.76 mg/100 g) as reported by Watada and Tran [43].

The vitamin B_2 content of the unfermented leaves extract was 0.28 mg/100 ml. The values for the fermented leaves extract increased from 0.53 to 1.27 mg/100 ml. The vitamin B_2 content values fell within the RDA values of the vitamins (1.2-1.7 mg) as reported by Hahn and Payne [44].

Mineral composition of fermented and unfermented seedless breadfruit (*A. communis*) leaf extracts

The mineral composition mg/100 ml of fermented and unfermented seedless breadfruit *(A. communis*) leaf extracts are shown in Table 5.

Magnesium content for the fermented leaf extract ranged from 1.48 to 2.34 mg/100 ml while unfermented leaves extract was 3.47 mg/100 ml. There was a general decrease in the magnesium with fermentation time. Stanton [45] also observed a reduction in the magnesium content of vegetables due to fermentation.

Calcium content of the unfermented leaf extract was 0.88 mg/100 ml. Fermented leaves extract values ranged from 0.53 to 0.95 mg/100 ml. There was no significant ($p<0.05$) difference among the treated and untreated samples. When compared with the calcium content of some vegetables such as *V. amygdalina* (bitter leaf) with 0.12 mg/100 g calcium content [46], *A. communis* seedless (breadfruit), had higher calcium content.

The iron content for the fermented leave extract value ranged from 0.19 to 0.76 mg/100 ml. Unfermented leaves extract value was 0.34 mg/100 ml. The iron content of the fresh sample (unfermented) was lower than that of spinach (0.6 mg/100 g) as reported by Mepha *et al*. [47].

Phosphorous content of the unfermented leaves extract was 5.57 mg/100 ml while the fermented leaves extract value ranged from 0.45 to 1.34 mg/100 ml. It was observed that unfermented sample had the highest phosphorous content value. Vitabase [48] reported that phosphorus is essential for the process of bone mineralization as well as a role in the structure of cellular membranes, nucleic acids, and nucleotide including adenosine triphosphate.

Microbiological count of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts

The results of the microbiological count of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts are shown in Table 6.

Table 3: Phytochemical composition of fermented and unfermented seedless breadfruit (*Artocarpus communis*) leaf extracts

Samples	Time (h)	Carotenoid (%)	Tanin (mg/100 ml)	Phenol (mg/ml)	Flavonoid (%)
Unfermented	0	$0.04^{a}\pm0.01$	$20.83^{b}\pm0.05$	$0.48^{c}\pm0.13$	$1.28^{c}\pm0.12$
Fermented	3	$0.09^{a}\pm0.01$	$30.04^{c}\pm1.21$	$0.08^{a}\pm0.03$	$0.84^{b}\pm0.09$
Fermented	6	$0.07^{a}\pm0.01$	$27.45^{c}\pm1.73$	$0.09^{a}\pm0.02$	$0.77^{ab}\pm0.00$
Fermented	9	$0.90^{a}\pm0.02$	$34.16^{d}\pm1.65$	$0.08^{a}\pm0.00$	$0.75^{ab}\pm0.01$
Fermented	12	$0.07^{a}\pm0.01$	$16.13^{a}\pm4.16$	$0.26^{b}\pm0.10$	$0.66^{a}\pm0.02$

Values are mean±standard deviation of triplicate determinations. Values bearing different superscripts within the same column are significantly different ($p<0.05$)

Table 4: Vitamin composition of fermented and unfermented seedless, breadfruit (*Artocarpus communis*) leaf extracts

Samples	Time (h)	Vitamin C	Vitamin E	Vitamin B_1	Vitamin B_2
Unfermented	0	$26.49^{a}\pm1.23$	$0.01^{a}\pm0.01$	$0.46^{a}\pm0.02$	$0.28^{a}\pm0.15$
Fermented	3	$15.54^{a}\pm0.44$	$0.06^{c}\pm0.02$	$0.46^{a}\pm0.01$	$0.80^{c}\pm0.08$
Fermented	6	$16.60^{c}\pm0.29$	$0.02^{ab}\pm0.01$	$0.49^{a}\pm0.00$	$0.53^{b}\pm0.11$
Fermented	9	$18.51^{b}\pm0.46$	$0.04^{bc}\pm0.02$	$0.46^{a}\pm0.01$	$1.27^{d}\pm0.07$
Fermented	12	$18.37^{b}\pm0.15$	$0.03^{ab}\pm0.01$	$0.47^{a}\pm0.02$	$0.57^{b}\pm0.07$

Values are mean±standard deviation of triplicate determination. Value bearing different superscripts within the same column are significantly different ($p<0.05$)

Table 5: Mineral composition of fermented and unfermented seedless breadfruit (*Artocarpus communis*) leaf extracts

Samples	Time (h)	Magnesium	Calcium	Iron	Phosphorus
Unfermented	0	$3.47^{c}\pm0.25$	$0.88^{a}\pm0.05$	$0.34^{b}\pm0.11$	$5.57^{c}\pm0.13$
Fermented	3	$1.63^{a}\pm0.09$	$0.95^{a}\pm0.02$	$0.76^{c}\pm0.08$	$1.34^{b}\pm0.50$
Fermented	6	$2.34^{b}\pm0.31$	$0.62^{a}\pm0.32$	$0.19^{ab}\pm0.03$	$0.47^{a}\pm0.03$
Fermented	9	$1.78^{a}\pm0.02$	$0.53^{a}\pm0.09$	$0.30^{a}\pm0.06$	$0.50^{a}\pm0.02$
Fermented	12	$1.48^{a}\pm0.07$	$0.64^{a}\pm0.18$	$0.21^{b}\pm0.06$	$0.45^{a}\pm0.15$

Values are mean±standard deviation of triplicate determinations value bearing different superscripts within the same column are significantly different ($p<0.05$)

Table 6: Microbiological count of unfermented and fermented seedless breadfruit (*Artocarpus communis*) leaf extracts

Samples	Time (h)	Total viable count (cfu/ml)
Unfermented	0	1.99×10^5
Fermented	3	14×10^5
Fermented	6	2.31×10^5
Fermented	9	1.73×10^5
Fermented	12	1.60×10^5

Values are means of triplicate determinations

Total viable count

The results obtained showed that there was decrease in growth of microorganisms with fermentation. This could be due to starvation as a result of loss of nutrient during the fermentation, which could lead to retarded growth. The total viable count of fermented sample for 12 hrs was the lowest followed by 9, 0, and 3 hrs while 6 hrs was the highest which had more growth of micro-organisms.

There were few microbial counts in the samples which could probably be due to the clean environment during processing, the raw materials used were kept in a refrigerator and clean containers were used to package the products after processing. This agreed with findings of Hobbs and Richard [49] that carried out food processing under aseptic condition from the raw materials to the finished products and observed few microorganisms in the food.

Isolation and biochemical characterization of organisms from unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts

Table 7 shows the isolation and biochemical characterization of organisms from unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts (Plates 1-5).

Microbiological status of fermented and unfermented *A. communis* extracts

The results for the isolation and characterization of the organism from the fermented and unfermented *A. communis* drink/extract are presented in Table 7. The data obtained constitute the profile of the strains and are also used for the identification of its genus. All strains reacted negatively to Gram staining (based on the bacillary morphology) apart from the *Bacillus* spp. The morphological, physiological, cultural, and biochemical tests/characteristics revealed a diversity of enteric bacteria which were classified based on the genera - *Klebsiella, Salmonella, Shigella, Escherichia, Staphylococcus, Pseudomonas,* and *Serratia* species. All the isolates were catalase positive, citrate positive and fermented mannitol, glucose, but there were positive delayed in sucrose and sorbitol respectively. All the isolates were non-spore formers except *Bacillus* spp. Furthermore, all the isolates reacted negatively to capsule stain apart from *Klebsiella* spp. There was evidence that these isolates (*Klebsiella, Shigella, Escherichia,* and *Bacillus* species) broke down lactose during fermentation while *Pseudomonas, Staphylococcus, Salmonella,* and *Serratia* species did not ferment lactose. These isolates are classified as enteric clinical species according to Brooks *et al.* [50]. Some isolates were also obtained from fermented vegetables (carrot, radish, and cucumber) according to Suja *et al.* [51].

The results obtained were in accordance to the physiological and biochemical characteristics described by Adams and Moss [52]. The identification results were confirmed by carbohydrate fermentation and assimilation profile obtained by API chart which correlated with Bergey's manual [64]. From the fermentation of selected carbohydrates, it showed that catabolism of these sugars resulted in the production of organic acids. Fermentation of these sugars could probably be due to the affinity of the fermenting microflora for the sugars. Both lactose and sucrose (as a disaccharide) reduced to simpler sugars unlike glucose (a monosaccharide) that was the starting point for Meyerhof-Embeden pathway. Glucose is the main channel for the breakdown of

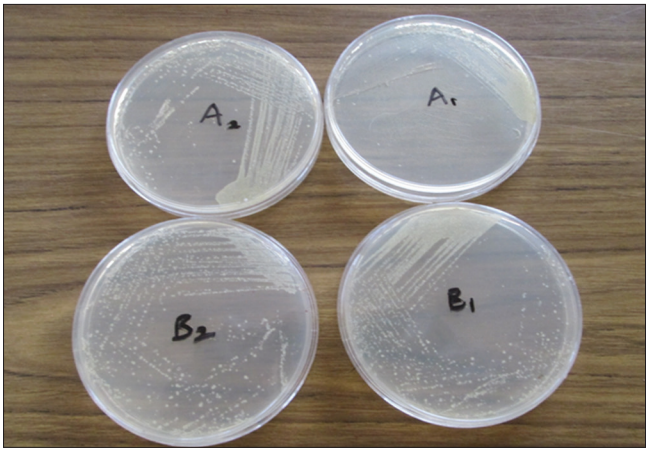

Plate 1: Isolation by streaking. A_1: Isolate from (0-h) unfermented *Artocarpus communis* extracts; A_2: Isolate from (0-h) unfermented *A. communis* extracts; B_1: Isolate from *A. communis* fermented for 3 hrs; B_2: Isolate from *A. communis* fermented for 3 hrs

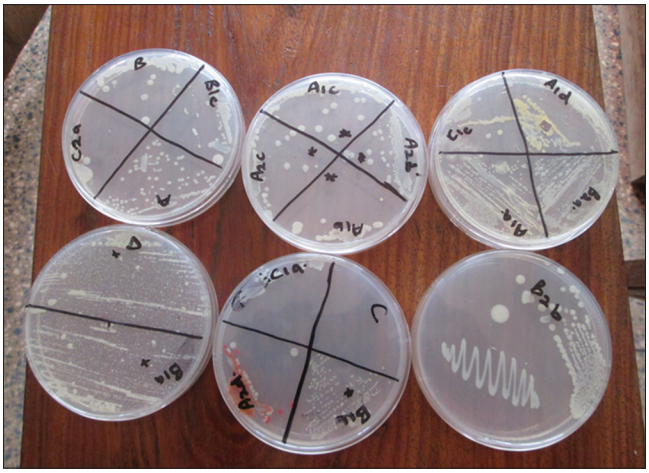

Plate 2: Isolated colonies from extracts. A, A_1a, A_1b, A_1c, A_1d, A_2c: Isolates from (0-h) unfermented *Artocarpus communis* extracts; B, B_1a, B_1b, B_1c, B_2a, B_2b: Isolates from *A. communis* fermented for 3 hrs; C, C_1a, C_1c: Isolates from *A. communis* fermented for 6 hrs

the monosaccharide in the muscles and microbial fermentation to the level of pyruvic acid [53]. It was also observed that the microorganisms took up oxygen through the mediation of flavoprotein oxidases. This was used to produce hydrogen peroxide and/or NADH produced during the dehydrogenation of sugars [52].

From the results obtained (Table 7), the fermented juices were observed to be safe for consumption except for the samples that contained *Salmonella* and *E. coli* as pathogens among others. However, there were isolates which were seen in the unfermented extracts. Motarjemi [54] identified that few organisms could be consumed in juices without having any adverse effects on the consumers. Ajayi and Adebolu [55], in their studies, revealed that micro-organisms play a substantial role in the spoilage of African breadfruit. From the fermented and unfermented extracts, the probable organisms were *Shigella*, *Salmonella*, *Staphylococcus*, *Klebsiella,* and *Serratia* species while the bacteria spoilage organisms were *E. coli*, Bacillus sp. and Pseudomonas sp. strains. Ajayiand Adebolu [55] stated that a strain of the Aspergillus sp., two strains of the Penicillium sp., and a strain of the Molinia sp. were isolated as fungal spoilage organisms. Bacillus sp. and Pseudomonas sp. strains were isolated as bacteria spoilage organisms. These species of micro-organisms were suspected after preliminary microscopy and biochemical tests were conducted

Table 7: Isolation and biochemical characterization of organisms from unfermented and fermented seedless breadfruit (*Artocapus communis*) leaf extracts

Colony morphology	Gram stain reaction	Spore stain reaction	Capsule stain reaction	Lactose fermentation	Manitol fermentation	Glucose fermentation	Sucrose fermentation	Sorbitol fermentation	Citrate test	Catalase test	Probable organisms	Samples
Circular shape, transparent, raised elevation, entire edge will mucoid consistency, smooth surface, uniform turbidity emulsification and no odour	-	-	+	+	+	+	+	+	+	+	*Klebsiella neumoniae*	UFo^{a}, F_3^{a}
Circular shape, transparent, raised elevation, entire edge with butyrous consistency, smooth surface, uniform turbidity emulsification and no odour	-	-	-	+	+	+	+	+	+	+	*Shigella* spp.	UFo^{b}, F_3^{d}
Circular shape, transparent, raised elevation, entire edge with mucoid consistency, smooth surface, uniform turbidity emulsification and no odour	-	-	-	-	+	+	+	+	+	+	*Pseudomonas aeroginosa*	UFo^{c}, F_3^{e}
Circular shape, transparent, flat elevation, irregular edge with granular consistency, rough surface, granular emulsification and no odour	+	+	-	+	+	+	+	+	+	+	*Bacillus* spp.	UFo^{d}, F_3^{f}
Circular shape, transparent, raised elevation, entire edge with butyrous consistency, smooth surface, uniform turbidity emulsification and no odour	-	-	-	+	+	+	+	+	+	+	*Escherichia coli*	UFo^{e}, F_3^{b}, F_6^{a}, F_9^{a}
Circular shape, transparent, raised elevation, entire edge with butyrous consistency, smooth surface, uniform turbidity emulsification and fishy odour	-	-	-	-	+	+	-	+	-	+	*Staphylococcus* spp.	UFo^{f}
Circular shape, transparent, raised elevation, entire edge with mucoid consistency, smooth surface, uniform turbidity emulsification and fishy odour	-	-	-	-	+	+	-	+	-	+	*Salmonella* spp.	UFo^{g}, F_3^{e}, F_6^{b}
Circular shape, insoluble orange pigment chromogensis, transparent, raised elevation, smooth surface with butyrous consistency, uniform turbidty emulsification and no odour	-	-	-	+	+	+	+	+	+	+	*Serretia marcescens*	UFo^{h}

UFo: Unfermented (a, b, c, d, e, f, g, h) Isolates from unfermented *A. communis* extracts, 3,6 and 9 time of fermentation, F_3 (a, d, e, f): Isolates from *A. communis* extract fermented for 3 hrs, F_6(a, b): isolates from *A. communis* extract fermented for 6 hrs, F_9 (a): Isolate from *A. communis* extract fermented for 9 hrs, +: Positive, -: Negative

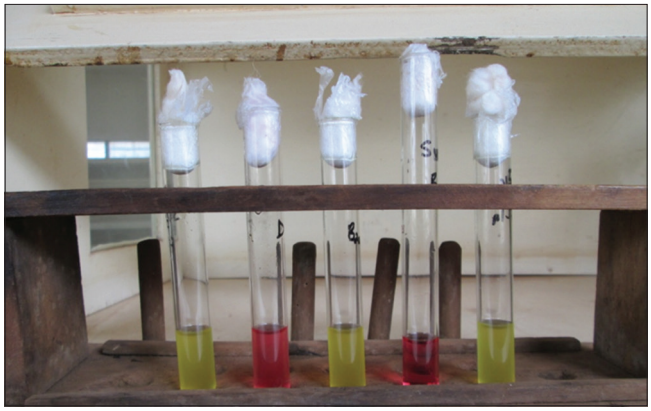

Plate 3: Sugar fermentation. Test tube 1 from right (A): Sugar fermented from (0-h) unfermented *Artocarpus communis* extracts; Test tube 2 (B): Sugar fermented from *A. communis* extract fermented for 3 hrs; Test tube 3 (B_2): Sugar fermented from *A. communis* extract fermented for 3 hrs; Test tube 4 (D): Sugar fermented from *A. communis* extract fermented for 9 hrs; Test tube 5 (C): Sugar fermented from *A. communis* extract fermented for 6 hrs

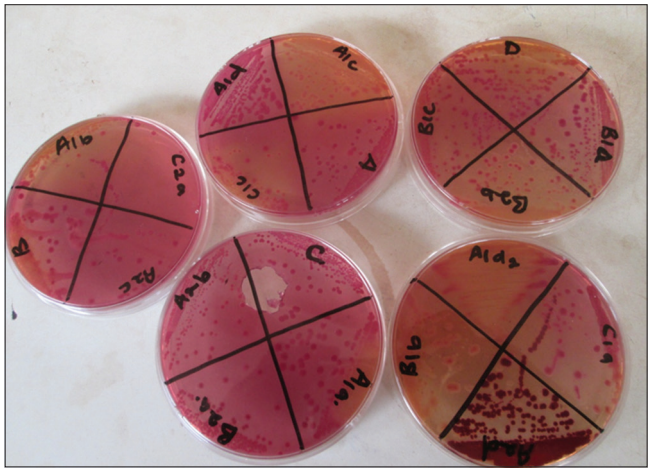

Plate 4: Identification of organisms using selective media (MacConkey agar, DCA: Desoxycholate citrate agar, cetrimide agar, starch agar). A, A_1a, A_1b, A_1c, A_1d, A_1d_2, A_2b A_2c, A_2d, A_2d: Isolates from (0-h) unfermented *Artocarpus communis* extracts; B, B_1a, B_1b, B_1c, B_2a, B_2b: Isolates from *A. communis* extracts fermented for 3 hrs; C, C_1a, C_1c, C_2a: Isolates from *A. communis* extracts fermented for 6 hrs; D: Isolate from *A. communis* extract fermented for 9 hrs

(Table 7). However, molecular analyses of the strains and pathogenicity test to reveal proliferation of the micro-organisms inoculated on the fresh sample indicating their spoilage activities to determine their genetic component and confirm their identity were not covered by the scope of this study. However, Omobuwajo and Wilcox [56] reported that micro-organisms which are associated with the spoilage of *A. communis* when on the field during planting include *Aspergillus* sp., *Rhizopus* sp., *Staphylococcus* sp., and *Mucor* sp. This has shown the spoilage micro-organisms involved during storage of the African breadfruit and their contributions to spoilage. The lactic acid fermentation with the bacteria have long been known and applied by humans for making different food stuffs [51]. Lactic acid bacteria (LAB) produce various compounds such as organic acids, diacetyl, hydrogen peroxide and bacteriocin or bactericidal proteins during lactic acid fermentation [57].

The high difference in the percentage crude protein shows that microorganisms made significant contribution to the deterioration

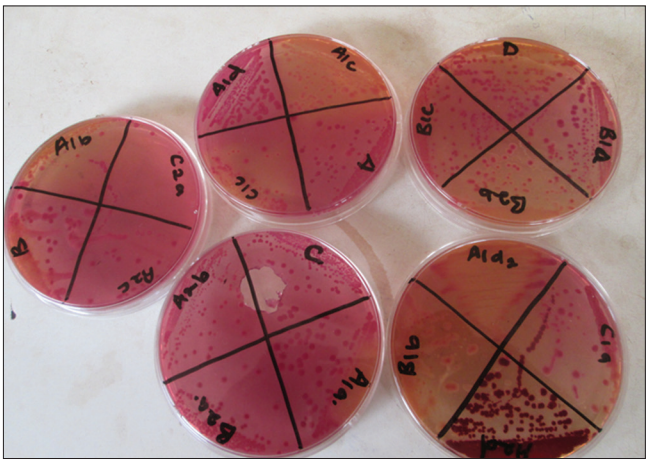

Plate 5: Identification of organisms using selective media (MacConkey agar, DCA: Desoxycholate citrate agar, cetrimide agar, and starch agar). A, A_1b, A_1c, A_2c, A_2d: Isolates from (0-h) unfermented *Artocarpus communis* extracts; B, B_1a, B_1b, B_1c, B_2b: Isolates from *A. communis* extracts fermented for 3 hrs; C, C_1a, C_2a: Isolates from *A. communis* extracts fermented for 6 hrs; D: Isolate from *A. communis* extract fermented for 9 hrs

of breadfruit. Although the African seedless breadfruit (*A. communis*, Forst) deteriorates due to its high respiration rate in storage, this study has revealed that deterioration rate becomes significantly higher with the presence of spoilage microorganisms. Provision of good storage conditions will substantially delay spoilage of breadfruit, the knowledge of which will contribute essentially to nutrition and economy of the Nigerian populace. Fermentation of the breadfruit into shelf stable extracts in this study could be exploited.

Sensory scores of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts

The sensory scores of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts are shown in Table 8. From the preliminary studies, the leaf extracts were fermented from 0 to 48 hrs. However, the samples fermented beyond 12 hrs were observed to have offensive odor and the taste was not preferable by the panelist. Therefore, the fermentation period was restricted to 0-12 hrs and the samples were stored at cold temperature (in the refrigerator at 4°C±2°C).

The color of different samples ranged from 6.87 to 7.50. The unfermented sample control (7.50) had the highest value in terms of color followed by fermented samples 9 hrs (7.30), 6 hrs (7.20), 3 hrs (7.13), and 12 hrs (6.87) which was the least. There was no significant ($p<0.005$) difference between the samples. This could be that the panelists most preferred the color of the unfermented sample probably because of its unique green color. This was in agreement with the study done by Karovieova and Kohajdova [58] on cabbage-carrot juices, for color, no differences were noticed as the color of the juices was in the range of good to very good.

The flavor of the different samples ranged from 5.97 to 6.97. Fermented samples for 12 hrs had the highest value (6.97) followed by 6 hrs (6.83), 9 hrs (6.80), and 3 hrs (5.97) while the unfermented 0-h had the scored of (6.73). There were no significant ($p<0.05$) difference between unfermented samples, fermented samples 6, 9, 12 hrs, except 3 hrs. It was observed that fermented sample for 12 hrs had the highest in flavor and most preferred by the panelists. This agreed with the study carried out by Larmond [59] that fermentation add flavor to foods.

The taste scores of the samples revealed that there was no significant ($p<0.05$) difference. The taste of different samples ranged from 6.23 to 6.97. Unfermented sample control (6.97) had the highest followed by fermented samples 6 hrs (6.83), 12 hrs (6.70), 9 hrs (6.30) and

Table 8: Sensory scores of unfermented and fermented seedless breadfruit (*A. communis*) leaf extracts

Samples	Time (h)	Color	Flavor	Taste	Aftertaste	Aroma	Appearance	Overall acceptability
Unfermented	0	7.50^{a}±0.86	6.73^{b}±1.05	6.97^{a}±0.89	7.03^{a}±1.40	6.73^{a}±1.35	7.03^{a}±1.56	7.53^{a}±0.90
Fermented	3	7.13^{a}±1.57	5.97^{a}±1.65	6.23^{a}±1.17	6.47^{a}±1.59	6.50^{a}±1.22	6.83^{a}±1.18	6.93^{a}±1.39
Fermented	6	7.20^{a}±1.42	6.83^{b}±1.26	6.83^{a}±1.21	6.77^{a}±1.52	7.00^{a}±1.39	7.10^{a}±1.63	7.33^{a}±1.45
Fermented	9	7.30^{a}±0.99	6.80^{b}±1.30	6.30^{b}±1.51	6.43^{a}±1.45	6.73^{a}±1.34	7.30^{a}±1.02	7.20^{a}±0.89
Fermented	12	6.87^{a}±1.66	6.97^{b}±1.40	6.70^{a}±1.37	6.93^{a}±1.23	6.60^{a}±1.35	7.07^{a}±1.46	7.13^{a}±1.14

Values are means±standard deviation of 30 panelists. Values bearing the same superscripts within the same row are not significantly ($p<0.05$) different

3 hrs (6.23), which was the least. This could be that the panelist most preferred the taste of the unfermented sample. This was in agreement with the sensory analysis done by Fayemi [60] on fresh "Nchuanwu" (Scent leaves) that the taste was rated (6.81) which was in the same range with unfermented sample.

The aftertaste of the different samples ranged from 6.43 to 7.03. The unfermented sample control (7.03) had the highest followed by fermented samples 12 hrs (6.93), 6 hrs (6.77), 3 hrs (6.47), and 9 hrs (6.43) which was the least. There were no significant ($p<0.05$) difference between the samples. This could be attributed to the fact that the panelists most preferred the aftertaste of the unfermented sample. This was also in agreement with the sensory quality carried out by Adubofuor *et al.* [61] on tomato juice and carrot juice that the aftertaste of unfermented sample were most preferred by the panelists.

Aroma scores of the samples revealed that there was no significant ($p<0.05$) difference. The aroma of the different samples ranged from 0.50 to 7.00. Unfermented sample control (6.97), fermented samples 6 hrs (7.00) had the highest followed by 9 hrs (6.73), 12 hrs (6.60) and 3 hra (6.50) which was the least. This could be also that the panelists most preferred the aroma of the fermented sample for 6 hrs. However, similar results were obtained by Arslan *et al.* [62] using caper leaves *Capparis spinosa* that fermentation between 5 to 6 hrs gives good aroma to the food.

The appearance of ranged from 6.83 to 7.30. Unfermented sample control (7.03), fermented samples 9 hrs (7.30), had the highest followed by 6 hrs (7.10), 12 hrs (7.07) and 3 hrs (6.83) which was the least. There were no significant ($p<0.05$) among the samples because all the samples possessed the same greenish color from the leaf pigment (chlorophyll). This was in agreement with the sensory scores carried out by Coleman (1990); on leaf vegetable juice (lettuce) that the appearance of the sample fermented between 8 and 10 hrs was most preferred by the panelist.

The overall acceptability of the different samples ranged from 6.93 to 7.53. The unfermented sample control (7.53) had the highest followed by fermented samples 6 hrs (7.33), 9 hrs (7.20), 12 hrs (7.13), and 3 hrs (6.93) which was the least. There were no significant ($p<0.05$) difference between the samples. This could also be attributed that the panelists most preferred the color of the fresh leaf (which is the control). This agreed with the research carried out by Dina *et al.* [63] that extracts from unfermented (fresh) leafy vegetable "ugu" (*T. occidentalis)* had the highest score (7.50) in overall acceptability which fall within the same range.

CONCLUSION

This research showed that vegetable juice can be produced from unfermented and fermented seedless breadfruit (*A. communis*) leaves. It was observed that the color and overall acceptability of the unfermented leaf extracts were most preferred by the panelists and had the highest ratings, respectively. Fermentation increased the tannin, carotenoid, alkaloid, vitamin (E and B_2), mineral (iron, calcium) contents and reduced the protein, fat, carbohydrate, phytate, oxalate, saponin, magnesium, and phosphorus contents. It was also observed that the samples fermented for 9 and 3 hrs had high moisture content. In some samples, there was no spoilage of micro-organisms. The microbial characterization of the probable micro-organisms (*Klebsiella* spp., *Shigella* spp., *Pseudomonas aeruginosa, Bacillus* spp., *E. coli, Staphylococcus* spp., *Salmonella* spp., and *Seretia marcescens*) was found in minimal amounts, in both unfermented and fermented samples, which might not be harmful to the body system.

Based on the findings, it would be necessary to assess the constituents of nutritional and medicinal value in the leaf of *A. communis*. There is also need to investigate the effect of fermentation on the individual constituents and their reactions in the leaf. This would provide vital data for the food processors, industrialists, nutrition workers, and consumers in the selection of the best period of fermentation for production of vegetable juice. Processing under aseptic condition should be adopted to reduce the microbial load of the products to prevent contamination, and in turn, infection/intoxication to the body when consumed. Genetic studies should be carried out to characterize the isolates.

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