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Microbiological Quality of Raw, Boiled and Fermented Breadnut Seed (*Artocarpus camansi*) - Used as Condiment

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Authors' contributions

This work was carried out in collaboration between the authors. Author AAN designed the study, performed the statistical analysis, wrote the protocol, and the first draft of the manuscript. Author OOO managed the analyses of the study and the literature searches. Author AAN supervised the research and interpreted the data and prepared the final manuscript draft. Both authors read and approved the final manuscript.

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ABSTRACT

Aims: The aim of the study was to investigate the microbial safety and quality assessment of processing breadnut (*Artocarpus camansi)* into boiled and fermented product for 72 hours at room temperature (30°C±2°C) as condiment.
Methodology: The raw seed was boiled with 0.07% salt as the boiled sample and fermented breadnut seeds were washed, boiled (2hrs), dehulled and wrapped in blanched plantain leaf. It was later boiled again for 2 hours, drained, cooled and allowed to ferment naturally for 72hrs while the raw sample serve as the control. The microbiological quality of the three samples was determined. Isolates were further characterized and identified base on cultural and biochemical characteristics.
Results: The mean total viable bacterial count of raw, boiled and fermented sample decreased from 4.22±0.31 to 1.04±0.16$\log_{10}$cfu/g. *Staphylococcus* count range from 3.21±0.13 to 1.04±0.07 $\log_{10}$cfu/g. Coliform count range from 2.31±0.43 to 1.13±1.06 $\log_{10}$cfu/g. Fungi count range from

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2.24±0.07 to 1.02±0.16 log_{10}cfu/g, respectively while there was no growth of *Salmonella* in the samples. There were significant differences in all the attribute rated for the three samples at ($P \leq .05$). Bacteria isolates were identified as *Bacillus subtilis, Bacillus laterosporus, Bacillus coagulans, Bacillus licheniformis, Bacillus pumilus, Micrococcus luteus, Micrococcus varians, Corynebacterium* sp, *Enterobacter* cloacae*, Escherichia coli*, *Staphylococcus aureus* and *Staphylococcus* sp. Fungi isolates were identified as *Rhizopus nigricans* and *Saccharomyces cerevisiae*. The predominant microbes in the samples are *Bacillus subtilis* and *Saccharomyces cerevisiae* which are beneficial microbes. The coliform, *Micrococcus* and *Staphylococcus* present in the 0 and 24hrs fermented seeds were completely eliminated in the 72 hrs fermented sample.

Conclusion: This research has proven that fermented breadnut seed can be used as condiment in soup because it consist of beneficial microorganisms which increases the nutritional value of food and helps to reduce food-borne diseases microbes that can be hazardous to health.

Keywords: Breadnut seed; quality; processing; boiling; fermentation.

1. INTRODUCTION

Breadnut (*Artocarpus camansi)* belongs to the Mulberry family Moraceae, has often been considered to be a seeded breadfruit species and is primarily grown for its nutritious seeds [1]. Breadnut is native to new guinea, Molucca and abundant in the Philippines [2]. The fruit has been neglected, underutilized and underdeveloped. Consequently, its utilization for a long time has been limited to vegetable stew with coconut milk and boiled seeds only by the Philippines [3]. In western Nigeria, the seed is consumed only as boiled samples. The uses of breadnut seeds is that mature seeds are boiled in salted water together with the shell (aril) and underlying membrane. Boiled or roasted breadnut seeds are delicious with a flavour resembling that of chest nut. Immature fruit are thinly sliced and boiled as a vegetable in soups or stews [4].

The fruit contains numerous seeds reported to be a good source of protein, carbohydrates, and minerals as documented by [4,5,6]. Increased cultivation and consumption of the seed in order to help alleviate nutritional deficiency in many of the developing areas of the world has been recommended by [6]. In addition, [1] enumerated the amino acids and fatty acids content of the seeds as follows; the seed was rich in leucine 392 mg/gN and phenylalamine 312 mg/gN but low in tryptophan 24 mg/gN and methionine 95 mg/gN while the principal fatty acid components in the breadnut seeds oil are palmitic 21.4%, linolenic 14.8% and oleic 12.4%.

However, breadnut fruit has a shelf life of 2-3 days after they are ripe and deteriorate rapidly [7,8]. Its highly perishable nature results to apparent rotten fruits during its season in the garden and the market [1]. They further pointed out that the seed needs to be processed to avoid spoilage and wastage during its season. The seeds can be preserved by processing or drying. Fermentation actually holds promising as a food processing method that can be applied to diversify the food usage of some under exploited plant foods [9]. In traditional fermentation processes, natural microorganisms are employed in the preparation and preservation of different types of food. These processes add to the nutritive value of foods as well as enhance flavor and other desirable qualities associated with digestibility and edibility [10].

Nigeria is endowed with a wide range of indigenously fermented foods and condiment [11] which are traditionally packaged with leaves [12]. Among such is IRU (Fermented African locust bean), Ogiri (fermented melon seed) etc. Despite the nutritive value of breadnut seeds, it had been reported to be an underutilized food source [13]. Although, various researches have been carried out on the nutrient composition and usefulness of breadfruit and breadnut as food [14] but no work has been done on the microbiological qualities of boiled and fermented product. This present work focuses on the evaluation of the microbial safety and quality of boiled and fermented breadnut seed as condiments.

2. MATERIALS AND METHODS

2.1 Sample Collection and Preparation

Breadnut seeds were collected from Sayedero market in the localities of Ilaro, Yewa South of Ogun State. The breadnut pulps were removed and the seed obtained were placed into a clean sterile container and taken to the laboratory for microbial analysis. The raw samples was

prepared by measuring 10 g of the fresh raw seed into the mortal and ground to form paste like solute that was commute to form a solution used for plating. The boiled samples were prepared by placing raw seeds into a clean and sterile pot with sterile water in a ratio 6:1 with 0.07% salt and boiled for 1 hr. Fermented samples of undehulled Breadnut seeds were properly washed, boiled for 2 hours, cooled and dehulled. The dehulled seeds of desire quantities (50 seeds per wrapped) were wrapped tightly in layers of blanched plantain leaves and pierced with fork. The wrapped cotyledons were latter boiled for 2 hours, removed from water and placed on a wire mesh to drain for 1 hour. The wrapped cotyledon was then left to ferment at the prevailing ambient temperature (28^oC) for 72 hours respectively for natural microflora to act on. At the end of the fermentation period, the seeds were pounded into paste. The paste was subsequently heated, dried and distributed into various polyethylene wrapping [10] as depicted in Fig. 1.

2.2 Preparation of Culture Media

The culture media used for this research are Nutrient Agar (NA), Eosin Methylene Blue Agar (EMB), MacConkey Agar (MAC), Baird Parkers Agar (BPA), Bismute Sulphite Agar (BSA) for bacterial analysis and Potato Dextrose Agar (PDA) for fungal analysis. The media were prepared and sterilized according to the manufacturer's specification [15].

2.3 Isolation and Identification of Micro-organisms

The raw, boiled and daily changes in the microbial population (cfu/g) of the fermenting breadnut seeds at 0, 24, 48 and 72 hours interval respectively were determined. Ten (10) grams of each breadnut samples was diluted in 90 ml of sterile distilled water in a conical flask to get the aliquot, a ten fold serial dilution was carried out. An aliquot of 1 ml from selected dilutions of each sample was inoculated aseptically into labelled triplicate agar plates of the media (NA, for total viable count, MAC for total coliform, BPA for *Staphylococcus* count, BSA for *Salmonella* count) using standard pour plate method and incubated at 37°C ±2°C for 24 to 48 hours. Potato Dextrose Agar was incubated at (28°C±2°C) for 3 to 5 days for isolation of fungi. Colonies were enumerated at the end of incubation period using digital colony counter (Gallenkamp England) [15,16]. Microbial colonies were counted and recorded. Isolates were preserved on appropriate agar slants stored at 4°C for further analysis. Presumptive isolates were further characterized and identified on the bases of colonial morphology, microscopic and biochemical characteristics to include; indole production, Voges-proskauer, citrate utilization, motility, spore stain, urease production, catalase, oxidase, coagulase, starch hydrolysis, fermentation of glucose, lactose, sucrose, maltose, xylose and galactose [17,18,19].

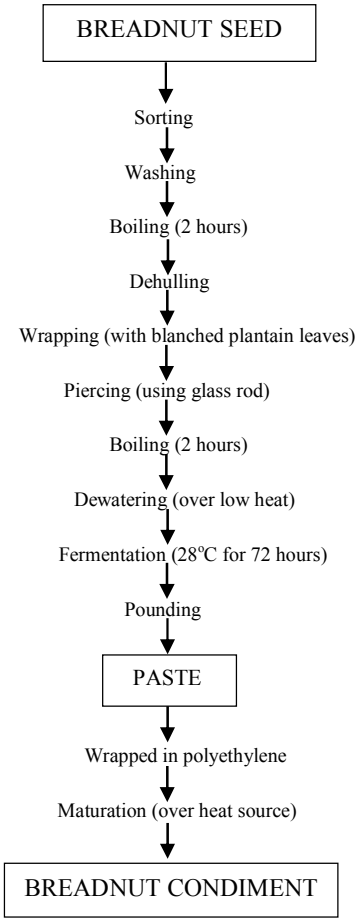

Fig. 1. Flow chart of breadnut condiment

2.4 Identification of Fungi Isolates

Identification of fungal isolates was done using lactophenol in cotton blue stain. The stained slides were examined with the aid of a microscope [20,21].

2.5 Statistical Analysis

All data obtained were subjected to statistical analysis of variance (ANOVA) using SPSS version statistical packages. Means were separated using DUNCAN Multiple Range Tests (DMRT) [22].

3. RESULTS

The results for microbial analysis of raw, boiled and fermented breadnut samples are presented in Table 1. The total viable bacteria count for raw, boiled and fermented samples ranges from 4.22±0.31 to 1.04±0.16 log_{10}cfu/g to 1.43 ±0.31 log_{10}cfu/g, this depicts that raw sample has the highest microbial load. The Staphylococcus count ranges from 3.21±0.13 log_{10}cfu/g to 1.04±0.07 log_{10}cfu/g to 1.63±0.23 log_{10}cfu/g for raw and fermented samples respectively. The result also show that raw sample has a highest *Staphylococcus* count and the 0hr fermented sample has the least count. No growth was observed for the boiled, 48 and72 hours fermented samples (i.e fermenting the seed for 72hrs reduces the pathogenic organisms that might cause food poisoning). Coliform count ranges from 2.31±0.43 log_{10}cfu/g to 1.16±0.11 log_{10}cfu/g to 1.70±0.37 log_{10}cfu/g for raw, boiled and 24hrs fermented samples respectively. This indicates that the raw sample has higher coli form count followed by 24 hrs fermented samples while the boiled sample recorded the least count. There was no growth of coli form at 48 hrs and 72 hrs fermented samples and no growth of *Salmonella* in the whole samples. The fungi count ranges from 2.24±0.07 log_{10}cfu/g to 1.21±0.06 log_{10}cfu/g to 1.02±0.16 log_{10}cfu/g respectively.

The result of the microscopic and biochemical characteristics of bacteria detected in the raw, boiled and fermented samples are shown in Table 2. From the result, three types of colonies were observed on the nutrient agar plate. The organisms on the agar plate which are creamy, yellow and pink in colour showed regular rod and cocci shape. The microbes isolated from the three samples was subjected to biochemical test to identify the suspected organism. Bacteria isolate were identified as *Bacillus subtilis*, *Bacillus laterosporus, Bacillus coagulans, Bacillus licheniformis, Bacillus pumilus, Micrococcus luteus, Micrococcus varians, Staphylococcus aureus, Staphylococcus* sp, *Corynebacterium* sp, *Enterobacter* cloacae *and Escherichia coli*.

The fungi isolates shows tiny creamy circular colonies with large spherical Budding cells and white filamentous hyphae with non septate spores. Fungi isolated were identified as *Saccharomyces cerevisiae and Rhizopus nigricans.* Successions of the isolated organisms in the raw, boiled and during the fermentation process of bread nut were presented in Table 3.

4. DISCUSSION

The microbiological safety and quality of raw, boiled and fermented products were evaluated in this study. The total viable bacteria counts shows a reduction in the counts from the raw samples to the fermented samples. Boiling the seed with

Table 1. Microbial analysis of raw, boiled and fermented breadnut seeds

Samples	(log10 cfu/g)				
	Total viable bacteria	***Staphylococcus* count**	**Coliform count**	***Salmonella* count**	**Yeast and mold count**
RAB	4.22 ± 0.31^{a}	3.21 ± 0.13^{b}	2.31 ± 0.43^{a}	NG	2.24 ± 0.07^{c}
BOB	1.36 ± 0.07^{c}	NG	1.16 ± 0.11^{c}	NG	1.21 ± 0.26^{a}
0FB	1.04 ± 0.16^{b}	1.04 ± 0.07^{c}	1.13 ± 1.06^{d}	NG	1.20 ± 0.06^{c}
1FB	3.22 ± 0.04^{c}	1.63 ± 0.23^{a}	1.70 ± 0.37^{b}	NG	2.13 ± 0.07^{c}
2FB	1.71 ± 0.03^{c}	NG	NG	NG	1.03 ± 0.01^{d}
3FB	1.43 ± 0.31^{a}	NG	NG	NG	1.02 ± 0.16^{b}

Values are Mean ±SD, Column with different superscripts are significantly (P ≤ 0.05) different.
Key: RAB – Raw Breadnut seed, BOB – Boiled Breadnut seed, 0FB-0 day Fermented Breadnut seed, 1FBD – One day fermented Breadnut seed (24 hrs), 2FBD – Two days fermented Breadnut seed (48hrs), 3FBD – Three days fermented Breadnut seed (72hrs). NG—No growth

Table 2. Microscopic and biochemical characteristics of bacteria isolates

Probable Identify	Colour	Gram rxn	Shape	Catalase	Oxidase	Indole	Motility	Coagulase	Spore	VP	Citrate	Urease	NO_3	Starch hydro	Sugar Fermentation					
															Glu	Suc	Lac	Xyl	Mal	Gal
Bacillus laterosporus	Cream	+	Rods	+	-	-	+	-	+	+	-	-	+	-	+	+	+	-	-	-
Staphylococcus aureus	Cream	+	Cocci	+	-	-	-	+	-	+	-	+	+	-	+	+	+	-	+	+
Enterobacter cloacae	Pink	-	Rods	+	-	-	+	-	-	+	+	+	+	-	+	+	+	+	+	+
Escherichia coli	Pink	-	Rods	+	-	+	+	-	-	-	-	-	+	-	+	+	+	+	+	-
Bacillus coagulans	Cream	+	Rods	+	-	-	+	-	+	+	+	-	+	+	+	+	+	+	+	-
Corynebacterium sp	Yellow	+	Rods	+	-	-	-	-	-	-	-	+	+	+	+	-	-	-	+	+
Bacillus subtilis	Cream	+	Rods	+	-	-	+	-	+	+	+	-	+	+	+	+	-	+	+	-
Staphylococcus sp	Cream	+	Cocci	+	-	-	+	+	-	+	-	-	+	-	+	+	+	-	+	+
Micrococus varians	Yellow	+	Cocci	+	+	-	-	-	-	-	+	-	+	-	+	+	+	+	+	-
Micrococus luteus	Yellow	+	Cocci	+	+	-	-	-	-	-	-	+	-	-	+	+	-	+	+	-
Bacillus pumilus	Cream	+	Rods	+	-	-	+	-	+	+	+	-	-	-	+	+	+	+	-	+
Bacillus licheniformis	Cream	+	Rods	+	-	+	+	-	+	+	+	-	+	+	+	+	-	+	+	+

Where +ve = positive, -ve = negative, Glu= Glucose, Suc = Sucrose, Lac = Lactose, Xyl = Xylose, Mal = Maltose, Gal = Galactose

Table 3. Succession of the isolates from breadnut samples

Microbes	RA	BO	OFB	1FB	2FB	3FB
Bacillus subtilis	+	+	+	+	+	+
Bacillus licheniformis	+	-	-	+	+	+
Bacillus laterosporus	+	+	+	+	-	-
Bacillus coagulans	-	+	-	+	+	+
Bacillus pumilus	-	-	-	+	+	-
Corynebacterium sp	+	-	-	-	-	
Staphylococcus aureus	+	-	+	-	-	-
Staphylococcus sp	+	-	+	+	-	-
Enterobacter cloacae	+	+	+	+	-	-
Escherichia coli	+	-	-	-	-	-
Micrococcus varians	-	-	-	+	-	-
Micrococcus luteus	-	-	-	+	+	-
Saccharomyce cerevisiae	+	+	+	+	+	+
Rhizopus nigricans	+	-	-	+	-	-

0.07% sodium chloride salt significantly reduces the microbial load to safe level from 4.22±0.31 to 1.04±0.16 $\log_{10}$cfu/g. An increase in the count was observed in the fermented seed from 0 and 24hrs. This represent the exponential phase of the growth of this microorganism [23] also reported a similar trend during the fermentation of breadfruit into gari. The increase could be due to availability of substrate prevalent conducive environment for the microorganism to metabolize the available substrate, while there was decrease in the 72 hrs fermented sample. The elimination of other organisms except *Bacillus* sp*,* and *Saccharomyces cerevisiae* might be due to the bacitracin that the *Bacillus* sp produced to inhibit their growth [24].

The *Staphylococcus* count recorded the highest value for the raw sample while the least count was recorded for the 0 hr fermented samples. For the 48 hrs and 72 hrs fermented samples, no count was observed (i.e. fermenting the seed for 3 days reduced the pathogenic organisms that might cause food poisoning). The presence of *Staphylococcus* sp in raw samples could be attributed to its wide spread in the environment. It could also be as a result of contamination from handlers. The presence of *Staphylococcus* sp is a normal flora of the human skin and mucosal membranes [25] but the boiled sample show pathogenic free and completely safe to consumed with same as that of 72 hrs fermented seed that show non-existence of *Salmonella, Staphylococcus* or coliform.

The result for coli form count of raw, boiled and fermented samples indicate that the raw sample has higher Coliform count followed by 24 hour fermented samples but the 48 and 72 hours fermented samples does not contain any Coli form organisms which is in accordance with [25,26]. That *Bacillus subtilis, Bacillus pumilis* and *Lactobacillus* sp were observed climax colonizer of fermented beniseed (Ogiri). The absence of the *Salmonella* growth in the whole samples make it safer. The occurrence of *Escherichia coli* and *Entero bacter* cloacae in raw and boiled samples indicate a contamination from the water activities in the environment. The occurrence of *Micrococcus* sp could be contamination from soil, dust, water and air. The *Bacillus* sp and *Micrococcus* sp as being the dominating organism in the 48 and 72hrs fermented seed have long been mentioned as causing proteolysis and lipolysis in fermenting seeds and grain with the release of free amino acids, fatty acids and glycerol [25].

The molds and yeasts isolated were *Rhizopus nigricans* and *Saccharomyces cerevisiae*. *Saccharomyces cerevisiae* were the dominating yeast in the fermented sample. This is in agreement with [10] who reported *S. cerevisiae* as the dominant microbes during the fermentation of melon seed to (ogiri) a local condiment. The presence of these microorganisms are not surprising as most of them are known to thrive in medium rich in fermentable substrates, such as carbohydrate

and sugar which often leads to the production of acid after fermentation.

Microbial succession of the isolate from Raw, boiled and fermented breadnut presented in Table 3 revealed that *Bacillus subtilis* occurs in all the samples*. B. laterosporus* and *Enterobacter cloacae* occur in raw, boiled, 0 and 24 hrs fermented samples. *Corynebacterium* sp and *Escherichia coli* occurs only in the raw, Staphylococcus occur in raw, 0 and 24hrs, *B coagulans* occurs in boiled, 24 ,48 and 72hrs fermented samples. *B pumilus* occurs only in 24 and 48 hrs fermented samples*. B licheniformis* occur throughout 72 hrs fermentation*. Micrococcus varians* occur in 24 hrs and *M. luteus* occur at 24 and 48 hrs fermented samples. *Saccharomyces cerevisiae* occurs in all the samples while *Rhizopus nigricans* were seen on the raw and 24hr fermented sample. From the microbial succession Bacillus species were the dominating microbes follow by *Enterobacter* in the raw and boiled samples. The dominant organisms during the fermentation periods were seen to be *Bacillus subtilis, B. licheniformis*, *B. coagulans* and *Saccharomyces cerevisiae.* This is in agreement with [27] who reported that quite a number of *Bacillus* species have been isolated from various fermented food condiments, although yeast and other bacteria were also seen, only part of them can be considered to play a substantial role in fermentation process.

Bacillus species were the dominating microbes in the fermented samples which are *Bacillus laterosporus* represents a particularly resistant probiotic microorganism. *Bacillus coagulans*; many references to use of this bacterium in humans exist, especially in improving the vaginal flora, improving abdominal pain and bloating inirritable bowel syndrome patients, and increasing immune response to viral challenges [28]. There is evidence from animal research that suggests that *Bacillus coagulans* is effective in both treating as well as preventing recurrence of *Clostridium difficile* associated diarrhea. One strain of this bacterium has also been assessed for safety as a food ingredient [29]. *Bacillus pumilus* generally show high resistance to environmental stresses. *Bacillus licheniformis* has proven to be an unexpected tooth decay fighter as it has the ability to cut through a layer of bacteria. *Bacillus subtilis* are known for production of useful antibiotics known as Bacitracin, due to its excellent fermentation properties, with high product yields (20 to 25 gram per litre) it is used to produce various enzymes, such as amylase and proteases [24,30].

Among the *Bacillus* species, *Bacillus subtilis* is the predominant bacteria responsible for the fermentation due to its high occurrence in the samples especially during the days of fermentation for 72 hours. This corroborate with the report of [25] tha*t Bacillus subtilis* are the predominant microbes in African locust bean. According to ICMSF [31] microbial standard for cashewnut, it should not contain any Coliform. *Staphylococcus count* should be between $1.0x10^2$ to $5.0x10^2$in 40g of sample. *Salmonella* count should be 10 in 50 g of sample. Comparing the result with the cashewnut standard, this shows that the 48 and 72 hrs fermented samples are safe for consumption.

5. CONCLUSION

The microbial load of raw breadnut seed is higher and unsaved to be consumed prior to processing either to be boiled or fermented in order to reduce the microbial load to safe level. Boiling with 0.07% sodium chloride for 2 hr reduces the microbial load. Fermented breadnut seed boil prior to fermenting for 72 hrs will completely eliminate pathogenic organisms and allows the *Bacillus* sp to thrive which can lead to improve the nutritional composition and aid easy absorption into the body system. This work revealed that *Bacillus subtilis* is the predominant microorganism involved in the samples most especially in the fermented samples. Hence boiling and fermentation process reduced the product with lower risk potential.

6. RECOMMENDATION

Boiled breadnut seed can be consume and the 72hours fermented breadnut seed might be used as condiment in soup just like African locust bean (IRU) since they both contain the same microorganism at the end of fermentation. Further research can be done on the molecular identification to strain level, nutritional composition and sensory evaluation of the 72hours fermented breadnut seed paste.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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