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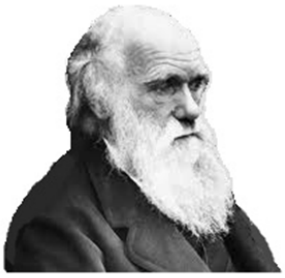

World News of Natural Sciences

An International Scientific Journal

WNOFNS 57 (2024) 59-67 EISSN 2543-5426

Analysis of the Bacterial Diversity During the Fermentation of Ukwa (*Treculia africana* Decne)

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ABSTRACT

African breadfruit (*ukwa*) is a special delicacy consumed widely in southeastern Nigeria. This work analyzed the microbial diversity associated with the traditional fermentation of *ukwa.* Matured *ukwa* fruit that fell from the *ukwa* tree were collected and allowed to ferment under controlled conditions in the laboratory. Fermenting samples were collected at 48-hour intervals and analyzed following standard microbiological standards. Changes in pH and moisture content were also monitored during each sampling interval. Results revealed that the Lactic acid bacteria count was highest on day 6 of the fermentation. A total of seven bacteria genera comprising *Bacillus, Lactobacillus, Staphylococcus, Escherichia coli, Proteus, Klebsiella, Pantoe,* and *Providencia* with *Bacillus* spp and lactic acid bacteria predominating the fermentation. The study also revealed a gradual decline in the pH and moisture until the last day of fermentation. This study has shown that the fermentation of ukwa is orchestrated by different groups of bacteria. The dominant organisms (*Bacillus* and *Lactobacillus* pp) can be developed as potential starter cultures to improve fermentation yield and enhance safety.

***Keywords*:** Ukwa, Treculia Africana, Fermentation, Bacteria, *Bacillus, Lactobacillus,* Lactic acid bacteria

1. INTRODUCTION

The African breadfruit (*Treculia africana*), popularly referred to as *ukwa* in southeastern Nigeria, is a huge evergreen tree that grows in tropical and subtropical climates. It is widely

(Received 19 August 2024; Accepted 23 September 2024; Date of Publication 28 September 2024)

dispersed over West and Central Africa and is a member of the Moraceae family [1]. *Ukwa* tree can reach a height of 30 meters with a stem width of up to 6 meters. The stem bark is grey in hue and produces white latex. The leaves are huge and dark green on the surface but lighter underneath. The seeds are widely consumed across socioeconomic strata in Southeastern Nigeria. They are the food of choice for special occasions such as weddings, burial ceremonies, age-grade celebrations, etc. [2].

Ukwa is a very nutritious food comprising protein (17–20%), carbohydrates (40%), oil (10%), and minerals, including magnesium, potassium, zinc, iron, calcium, sodium, copper, and vitamins [3]. It makes a significant contribution to dietary intake and help ameliorate seasonal food insecurity [1, 4]. In addition to the nutritional quality of the seeds, the pods, leaves, and roots are used extensively in traditional medicine. The leaves are exceedingly abundant in carbohydrates, phytochemicals (such as flavonoids, phenols, cardiac glycosides, and anthraquinones), and minerals. These components augment its antioxidant, antibacterial, and wound-healing characteristics. The stem bark extract is employed as a treatment for coughing and possesses antibacterial characteristics. The root extracts, both aqueous and ethanolic, have antihyperglycemic effects and also inhibit the progression of secondary problems associated with type 2 diabetes [5].

Traditionally, the heavy, large fruit of *ukwa* is not harvested but allowed to ripen and drop from the tree. The fruit is processed using either the fresh extraction process or the fermentation method, with the latter preferred because of the ease of extraction after fermentation. For the extraction method, a sharp kitchen knife is used to remove the spongy pulp in order to extract the seeds. In the fermentation method, the seeds are heaped together and allowed to ripen, after which the seeds are extracted using repeated washing to remove the pulp and mucilage. [5, 6].

The seeds are dehulled, roasted, and snacked in combination with palm kernel (Elais guineensis seed) or coconut (*Cocus nucifera*). Roasted dehulled seeds are frequently sold on roadsides in South East Nigeria. Fresh un-dehulled seeds can be blanched in boiling water for 5-10 minutes, dehulled, cooked to a soft consistency, and consumed as porridge or blended with cereals like rice yam, sorghum, and maize. Additionally, the dehulled seeds can also be dried and processed into flour and used for baking or thickening soups [5-7].

According to [8 & 9], the fermentation process is essential for the development of peculiar flavour, texture, and nutritional quality of African breadfruit. Several species of bacteria, including *Micrococcus, Lactobacillus, Proteus mirabilis*, *Klebsiella pneumoniae,* and *Staphylococcus aureus,* have been isolated from samples of African breadfruit. [6, 10]. However, there is still a paucity of information on the diversity of bacteria associated with the fermentation of *ukwa.* Therefore, this research aims to investigate the bacterial diversity associated with the traditional fermentation of *ukwa*.

2. MATERIALS AND METHODS

2. 1. Sample Collection and Fermentation Process

Matured *ukwa* fruits were obtained from a farm in Orodo, Imo State, Nigeria, and transported to the Microbiology Laboratory of Imo State University, Owerri, in sterile polythene bags. The fruits were placed on a clean table without cover to allow fermentation over a 10-day period.

2. 2. Isolation and Enumeration of Bacteria

10g of the fermenting *ukwa* was mixed with 90 ml of buffered peptone water and serially diluted using 0.1% peptone water. Samples were taken at 48hrs intervals until the end of fermentation by day 10. Aliquots of 0.1 ml were plated out onto Standard Plate Count Agar (Oxoid, UK), MRS Agar (Oxoid), and MacConkey Agar (Oxoid, UK). The plates were incubated at 37 °C for 24 hours, while MRS plates were incubated anaerobically for 48 hours. After incubation, colonies were counted using a colony counter, and only plates with 2 to 10 colonies were selected for further analysis. Isolates were purified by streaking onto fresh plates of Standard Plate Count Agar, MRS Agar, and MacConkey Agar.

2. 3. Physicochemical Parameters

The pH and moisture content were also determined at each sampling interval according to the method described by [11]. Each measurement was performed in triplicate to ensure the accuracy and reliability of the results.

2. 4. Identification of bacteria isolates

Isolates were identified using a combination of cultural, morphological, and biochemical characteristics. Biochemical tests such as indole, citrate utilization, motility, oxidase, urease, Methyl red, Voges-Proskauer, spore stain, growth at 15 °C, growth at 45 °C, growth at pH 4.5, sugar fermentation test, hydrogen sulfide production, and Coagulase were carried out

3. RESULTS

The result of the enumeration of the microorganisms revealed a slight increase in total bacteria and lactic acid bacteria counts at the initial days of retting (days 0-2). At the same time, coliforms were first detected on day two. A significant increase in total bacteria and lactic acid bacteria counts was observed as retting proceeded, particularly on day six, where the total bacteria count reached 3.6×10^4 CFU/g and lactic acid bacteria peak at 5.1×10^4 CFU/g. After day 6, there was a decline in total bacteria and lactic acid bacteria counts by day 10. Coliform counts also fluctuated but remained relatively low compared to the peak at day 6 (Table 1)

Table 1. Enumeration of bacteria from fermented *ukwa* (CFU/g).

Fermentation time (day)	Total bacteria count	Coliform count	Lactic acid bacteria
0	1.0×10^3	-	-
2	1.4×10^3	1.2×10^2	1.6×10^3
4	1.8×10^3	1.9×10^2	2.8×10^3
6	3.6×104	1.3×10^3	5.1×10^4

8	2.8×10^3	1.0×10^3	4.2×10^4
10	2.6×10	1.2×10^2	3.6×10^3

Table 2. Percentage Dominance of bacteria isolated from fermented *ukwa*

Isolate	No of occurrence	Percentage
Bacillus sp	8	36.3
Escherichia coli	3	13.64
Staphylococcus aureus	2	9.01
Klebsiella pneumoniae	3	13.64
Proteus	1	4.55
Providencia	1	4.55
Pantoe sp	1	4.55
Lactobacillus sp	3	13.64

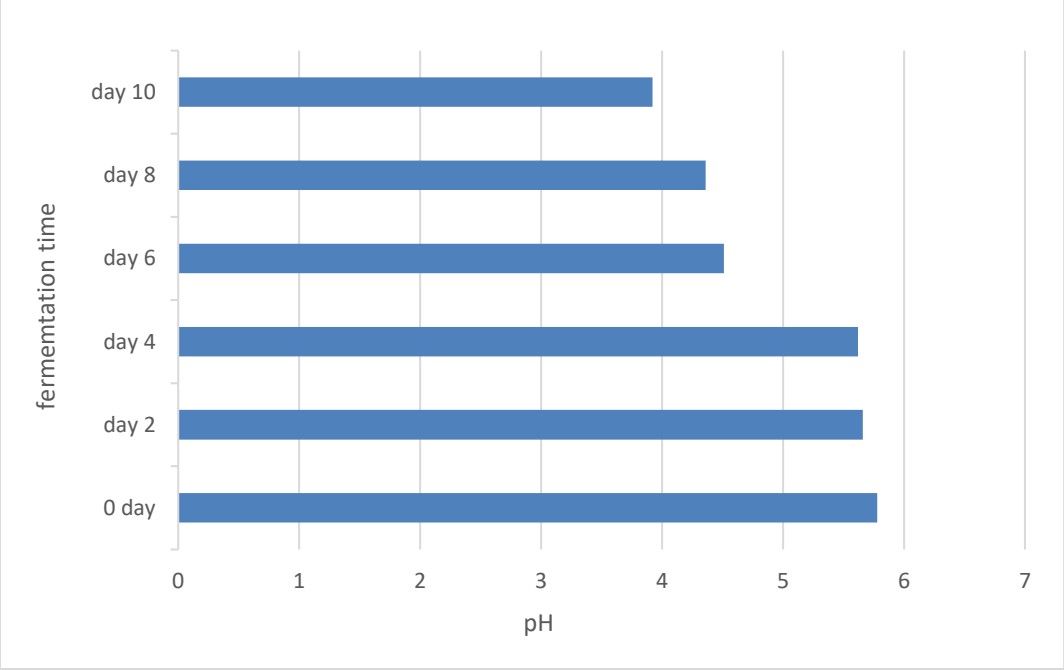

Figure 1. pH changes during the fermentation of *ukwa*

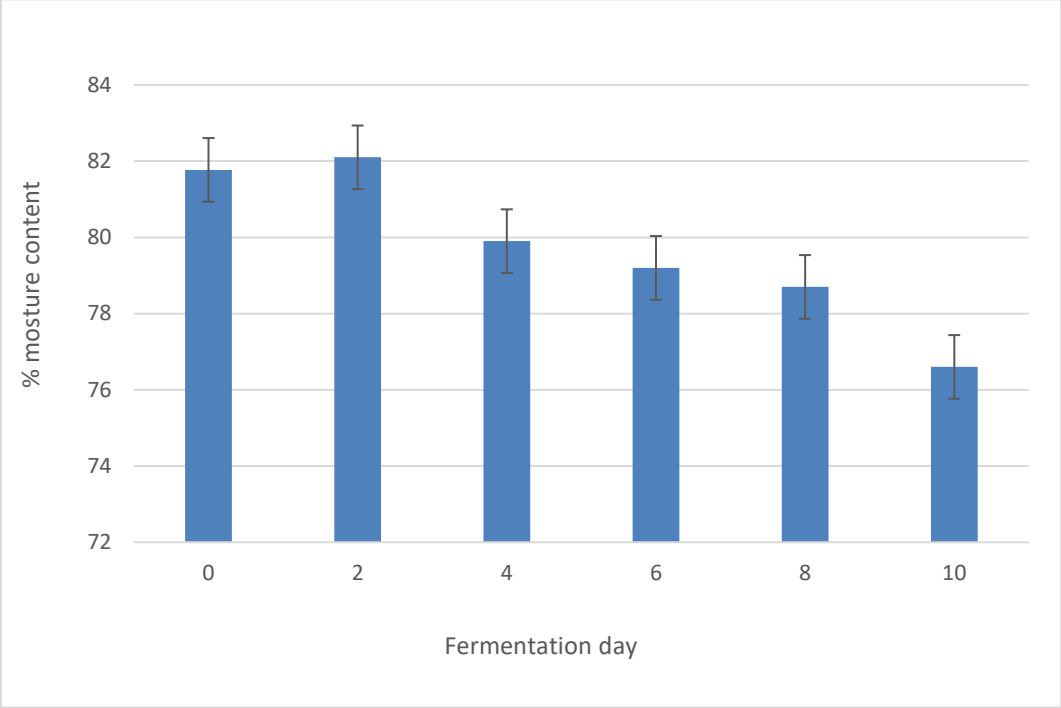

Figure 2. % changes in moisture content during fermentation of *ukwa*

There was a clear pattern of decreasing pH and moisture content throughout the period, with 3.92 and 76.6%, respectively, on day ten. (Figures 1 and 2). Preliminary identification based on morphological and biochemical characteristics reveals isolates as a genus of *Bacillus, Lactobacillus, Staphylococcus, Escherichia coli, Proteus, Klebsiella, Pantoe*, and *Providencia*. (Table 2). The result from Table 2 showed that *Bacillus* sp was the most dominant species (36.3%), followed by *Escherichia coli*, *Klebsiella pneumonia*, and *Lactobacillus* sp, which all have similar occurrence rates (13.64%). The remaining species had lower occurrences, indicating less dominance in the sample.

Table 2. Morphological and biochemical characteristics of bacteria isolates

Cell shape	Gram reaction	VP	Catalase	H_2S	Coagulase	Growth at pH 4-5	Oxidase	Urease	Citrate	Growth at 15 °C	MR	Indole	Glucose	Growth at 45 °C	Lactose	Probable organisms
Rod	-	-	+	-	-	N	-	+	+		+	-	+	N	+	*Klebsiella pneumoniae*
Rod	+	-	-	+	-	N	-	+	-		+	-	+	N	-	*Bacillus* spp
Rod	-	-	+	+	-	N	-	+	+		+	-	+	N	-	*Proteus* spp
Rod	+	-	+	+	-	N	-	+	-		+	-	+	N	-	*Bacillus* spp

Rod	-	N	+	-	-	N	-	-	-		+	+	+	N	+	*Escherichia coli*
Cocci	+	-	+	-	+	N	-	+	+		+	-	+	N	-	*Staphylococcus* spp
Rod	-	-	+	+	-	N	-	-	+		+	-	+	N	-	*Pantoe* sp
Rod	+	-	+	+	-	N	-	+	+		+	-	+	N	+	*Bacillus* spp
Rod	-	-	+	+	-	N	-	+	-		-	-	+	N	-	*Providencia* spp
Rod	+	-	-	-	-	+	-	N	N	+	+	-	+	-	+	*Lactobacillus* spp
Rod	+	-	-	-	-	+	-	N	N	+	+	-	+	-	+	*Lactobacillus* spp

Key: N: not tested +: positive, -: negative

4. DISCUSSION

The African breadfruit seed is a highly regarded culinary delight of the people of southern Nigeria. The fermentation South East Nigeria. According to [7 & 8], intricate microbial fermentation is responsible for the characteristics, taste, texture, flavour, and nutritional composition of African fermented foods [6, 7]. The result of this study has shown that the traditional fermentation process for *ukwa* is orchestrated by different genera of bacteria. The enumeration of microorganisms showed that lactic acid bacteria (LAB) had the highest count (5.1×10^4 CFU/g). Other researchers have also reported the dominance of lactic acid bacteria in many Indigenous fermented products and their crucial role in flavour development and preservation [12 and 13].

A total of eight bacteria genera comprising *Bacillus, Lactobacillus, Staphylococcus, Escherichia coli, Proteus, Klebsiella, Pantoe*, and *Providencia* were identified with *Bacillus* and *Lactobacillus* predominating [10] also reported *Bacillus, Lactobacillus, Micrococcus, Enterobacter*, and *Staphylococcus*, with *Bacillus* sp and *Lactobacillus* spp constituting the predominant microflora in their study. [14] also identified *Micrococcus* sp, *Lactobacillus fermentum, Bacillus subtilis,* and *Streptococcus* sp from their research.

According to [14], *Bacillus* is noted for its ability to produce enzymes extracellularly, including amylases, proteases, and lipases. These enzymes can degrade complex carbohydrates, proteins, and lipids to create simpler molecules that improve flavour and digestibility. This enzymatic breakdown is important in the fermentation of *ukwa* because it enhances its organoleptic properties. Pectinases, another enzyme produced by some species of *Bacillus*, have been associated with the liquefaction of plant tissues and also play an active role in the disintegration of the pulp during fermentation [10]. *Bacillus subtilis* has been implicated as one of the most important organisms in the retting process, as it has been shown to have a significant role in the fermentation of carbohydrate materials [15 & 16].

The presence of lactic acid bacteria (LAB), specifically *Lactobacillus* species, is significant since they are recognized for their contribution to the fermentation process via the production of lactic acid. Lactic acid enhances the taste and prolongs the shelf life of fermented products [1, 10]. The production of lactic acid and other byproducts also augments the sensory characteristics of *ukwa*, hence increasing its palatability [2].

Lactobacillus species have been heavily implicated in the fermentation process of many Nigerian fermented products, including *ogi*, *fufu*, *garri*, etc. [8-19].

In addition, several species of *Lactobacillus* are known for their probiotic characteristics with attendant improvement in gut health and immune system functioning. [20]. The result of the study also reveals that the duration of fermentation has a substantial impact on the dynamics of the microbial population, as evidenced by a well-defined rise in LAB counts during the fermentation period. This trend is corroborated by the works of [10, 14].

The pH and moisture content changes observed during fermentation are an indication of the metabolic activities of the microbiota involved. A gradual decline in pH from 5.78 to 3.92 was observed over the fermentation period (Figure 1). This decline is associated might be associated with the production of organic acids, primarily lactic acid, which not only contributes to the preservation of the product but also affects the overall flavour profile [2]. Although *Escherichia coli* and *Klebsiella* spp are gut microbes, their presence in the fermentation of *ukwa* raises concerns about food safety [21]. Some species of *Escherichia coli* and *Klebsiella* have the potential to cause gastroenteritis, diarrhea, urinary tract infections, endocarditis, and meningitis, especially in immunocompromised patients [22].

5. CONCLUSION

The study has revealed that the fermentation of *ukwa* involves the activities of different groups of bacteria. The result of the study also shows that *Bacillus* pp and *Lactobacillus* species are the dominant microflora. These organisms could be developed as starter cultures to improve the fermentation of *ukwa*. However, additional studies that are focused on isolating and evaluating the technological properties of these dominant organisms are crucial before their application as a starter culture.

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