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Comparative Bacteriological Analysis of Fermented and Unfermented Ugba (*Pentaclethra macrophylla*)

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ABSTRACT

Ugba, a traditional fermented condiment produced from African oil bean seeds (*Pentaclethra macrophylla*), is widely consumed in southeastern Nigeria. The fermentation process enhances the sensory properties and nutritional quality of the product; however, traditional processing methods may expose the food to microbial contamination. Understanding the bacteriological differences between fermented and unfermented Ugba is therefore important for evaluating its microbiological quality and potential food safety risks. This study comparatively assessed the bacteriological characteristics of fermented and unfermented Ugba in order to determine microbial populations associated with the fermentation process. Samples of fermented Ugba and unfermented African oil bean seeds were obtained from local market vendors and analyzed using standard microbiological procedures. Serial dilution and spread plate techniques were used to determine total heterotrophic bacterial counts and coliform counts. Bacterial isolates were identified based on colonial morphology, Gram staining, motility tests, and biochemical characterization including catalase, oxidase, citrate utilization, indole, methyl red–Voges Proskauer, and coagulase tests. The results showed higher total heterotrophic bacterial counts in fermented Ugba samples (mean 4.42 log₁₀ CFU/g) compared with unfermented samples (mean 4.01 log₁₀ CFU/g), indicating increased microbial activity during fermentation. Coliform bacteria were detected in some samples, with fermented Ugba showing higher counts (mean 4.38 log₁₀ CFU/g) than unfermented samples (mean 3.95 log₁₀ CFU/g). Identified bacterial isolates included *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., and *Staphylococcus aureus*, with *E. coli* occurring most frequently.

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The study demonstrates that fermentation increases microbial populations in Ugba while the presence of coliform and pathogenic bacteria suggests possible contamination during traditional processing. Improved hygienic practices and controlled fermentation methods are therefore recommended to enhance the microbiological safety of this widely consumed traditional food condiment.

Keywords: Ugba, African oil bean seed (*Pentaclethra macrophylla*), Fermentation, Bacteriological assessment, Food safety, Traditional fermented foods, Microbial contamination.

1. INTRODUCTION

Fermentation represents one of the earliest technologies developed by humans for the transformation and preservation of food. Through the metabolic activities of microorganisms such as bacteria, yeasts, and molds, fermentation converts complex substrates into simpler compounds that improve food flavor, aroma, digestibility, and shelf stability. In many parts of the world, particularly in developing countries, fermented foods constitute an important component of daily diets and contribute significantly to nutritional security and cultural culinary traditions (Holzapfel, 2002; Tamang *et al.*, 2016). Beyond preservation, fermentation can enhance the bioavailability of nutrients, reduce anti-nutritional factors, and generate beneficial bioactive compounds that contribute to improved human health (Marco *et al.*, 2017).

Across Africa, numerous indigenous fermented foods are produced from cereals, legumes, seeds, and tubers through spontaneous fermentation processes. These processes depend largely on naturally occurring microorganisms present in raw materials or the surrounding environment. The microorganisms involved in these fermentations drive a series of biochemical transformations that alter the chemical composition and microbiological characteristics of the food matrix (Olasupo *et al.*, 2016). As fermentation progresses, microbial enzymes degrade macromolecules such as proteins and carbohydrates, resulting in the formation of compounds responsible for the distinctive sensory attributes of traditional fermented foods.

One of the most widely consumed fermented seed condiments in southeastern Nigeria is Ugba, which is produced from the seeds of the African oil bean tree (*Pentaclethra macrophylla* Benth). Ugba occupies a significant place in the traditional diet of the Igbo people and is commonly consumed either as a delicacy or as a flavoring ingredient in soups and salads. The traditional preparation of Ugba involves several processing stages including prolonged boiling of the seeds, removal of the seed coats, slicing of the cotyledons into thin strips, repeated washing, and subsequent fermentation at ambient temperature for several days (Ogueke *et al.*, 2010). These processing steps initiate microbial activity that ultimately leads to the development of the characteristic taste, texture, and aroma associated with the fermented product.

Previous investigations into the microbiology of Ugba fermentation have demonstrated that the process is largely dominated by *Bacillus* species, particularly *Bacillus subtilis*. These organisms play a crucial role in the fermentation process through the production of extracellular enzymes that facilitate the breakdown of complex proteins into smaller peptides and amino acids. This proteolytic activity contributes significantly to the development of the distinctive flavor and improved digestibility of fermented African oil bean seeds (Ahaotu *et al.*, 2013; Olasupo *et al.*, 2016). Molecular studies have further confirmed the predominance of *Bacillus* species during Ugba fermentation and have highlighted their importance in ensuring the stability and quality of the final product (Ahaotu *et al.*, 2013).

In addition to microbial fermentation, the thermal processing steps involved in the preparation of Ugba have also been shown to influence the chemical composition of the seeds. Heat treatment softens the cotyledons and facilitates the removal of anti-nutritional factors while preparing the substrate for microbial colonization during fermentation (Enujiugha and Akanbi, 2005). Subsequent microbial activity during fermentation leads to further biochemical changes, including the reduction of anti-nutritional compounds and the enhancement of nutrient availability. These transformations contribute to the nutritional value and acceptability of Ugba as a traditional food condiment.

Despite its cultural and nutritional importance, Ugba production is largely carried out through traditional methods that often lack standardized hygienic controls. Fermentation typically occurs under household or small-scale conditions where environmental microorganisms may inadvertently contaminate the product during processing, handling, or storage (Okorie and Olasupo, 2013). Such conditions may allow the introduction or proliferation of undesirable microorganisms, which could affect both the safety and quality of the fermented product. Consequently, understanding the microbial composition of Ugba and the changes that occur during fermentation remains an important aspect of ensuring its microbiological safety.

Another important consideration is the difference between the microbial communities associated with fermented Ugba and those present in the unfermented African oil bean seeds. While fermentation encourages the growth of specific fermentative microorganisms that contribute to desirable biochemical transformations, unfermented seeds may harbor a diverse range of environmental bacteria that could influence product quality and safety. Comparative bacteriological analysis of fermented and unfermented Ugba is therefore essential for understanding microbial dynamics during the fermentation process and for identifying potential microbial hazards associated with traditional production practices.

Although previous studies have investigated the fermentation process of African oil bean seeds and the microorganisms involved, limited studies have directly compared the bacteriological profile of fermented and unfermented Ugba under similar conditions. Such comparative information is important for understanding microbial changes associated with fermentation and evaluating potential food safety risks.

Given the widespread consumption of Ugba in southeastern Nigeria and its importance as a protein-rich fermented condiment, there is a continuing need to evaluate its microbiological characteristics. Such studies provide valuable information for improving hygienic processing methods, enhancing product safety, and supporting the development of controlled fermentation approaches that preserve the nutritional and cultural significance of this indigenous food.

Therefore, the present study aimed to conduct a comparative bacteriological assessment of fermented and unfermented Ugba, with the objective of identifying the bacterial populations associated with both forms of the product and evaluating their potential implications for food safety and quality.

2. MATERIALS AND METHODS

2.1. Sample Collection

Fermented Ugba (*Pentaclethra macrophylla*) samples were purchased from vendors selling ready-to-eat Ugba in a local market, while unfermented African oil bean seeds intended for Ugba production were obtained from the same vendors prior to fermentation. Samples were aseptically collected into sterile containers and transported to the Microbiology Laboratory for bacteriological analysis.

2.2. Preparation of Culture Media

Culture media used in this study included Nutrient Agar, MacConkey Agar, Plate Count Agar, Simmons Citrate Agar, and Peptone Water. Media were prepared according to the manufacturer's instructions and sterilized by autoclaving at 121 °C for 15 min. Sterile media were poured into Petri dishes and allowed to solidify before use. All glassware and equipment were sterilized using standard microbiological procedures (Cheesbrough, 2006).

2.3. Sample Preparation and Serial Dilution

Ten grams of each sample were aseptically homogenized in 90 mL of sterile distilled water to obtain the initial suspension. Serial ten-fold dilutions were prepared using sterile diluent.

2.4. Enumeration of Bacteria

Total heterotrophic bacterial counts were determined using the spread plate method on Nutrient Agar. Aliquots (0.1 mL) of appropriate dilutions were inoculated onto sterile plates and incubated aerobically at 37 °C for 24 h. Colonies were counted and expressed as colony forming units per gram (CFU/g). Total coliform counts were determined using MacConkey Agar following the same procedure and incubation conditions. Selective isolation of *Salmonella* and *Shigella* species was carried out using Salmonella–Shigella agar and incubated at 37 °C for 24 h. Distinct colonies were purified by repeated sub-culturing on Nutrient Agar.

2.5. Identification of Bacterial Isolates

Bacterial isolates were identified based on colonial morphology, Gram staining, motility test, and biochemical characterization. Biochemical tests performed included catalase, oxidase, citrate utilization, indole production, methyl red–Voges Proskauer (MR–VP), urease, coagulase, and carbohydrate fermentation tests using standard microbiological methods (Cheesbrough, 2006; Prescott *et al.*, 2005).

2.6. Statistical Analysis

Data obtained from microbial counts were converted to logarithmic values (log₁₀ CFU/g) prior to analysis. Mean values and standard deviations were calculated. Differences between fermented and unfermented samples were analyzed using Student's t-test, and statistical significance was considered at $p < 0.05$.

3. RESULTS

3.1. Total Heterotrophic Bacterial Counts of Ugba Samples

The total heterotrophic bacterial counts of ugba samples before and after two days of fermentation are presented in Table 1a. The bacterial population of the unfermented samples ranged from 3.95 to 4.08 log₁₀ CFU/g, whereas fermented samples exhibited higher values ranging from 4.30 to 4.50 log₁₀ CFU/g. Statistical analysis using Student's *t*-test showed that the increase in bacterial counts following fermentation was statistically significant ($p < 0.05$) as presented in Table 1b.

Table 1a. Total heterotrophic bacterial counts of ugba samples before and after fermentation.

Sample	THBC (CFU/g)	Log ₁₀ CFU/g
AUF	9.6×10^3	3.98
BUF	1.2×10^4	4.08
CUF	1.1×10^4	4.04
DUF	9.0×10^3	3.95
AF	3.15×10^4	4.50
BF	2.49×10^4	4.40
CF	2.0×10^4	4.30
DF	3.0×10^3	3.48

Key

AUF–DUF = Unfermented ugba samples

AF–DF = Ugba samples

THBC = Total heterotrophic bacterial count

Table 1b. Mean microbial counts.

Sample group	Mean (log ₁₀ CFU/g)	Standard deviation
Unfermented ugba	4.01	±0.05
Fermented ugba	4.42	±0.09

3.2. Total Coliform Counts of Ugba Samples

The distribution of coliform bacteria in ugba samples is presented in Table 2. Coliform bacteria were detected in only one of the unfermented samples, while all fermented samples showed detectable coliform populations. The microbial load of fermented samples ranged from 4.15 to 4.56 log₁₀CFU/g. Statistical analysis indicated that the difference between the two sample groups was significant ($p < 0.05$) as presented in Table 2b

Table 2a. Total coliform counts of fermented and unfermented ugba samples.

Sample	TCC (CFU/g)	Log₁₀ CFU/g
AUF	ND	—
BUF	9.0×10^3	3.95
CUF	ND	—
DUF	ND	—
AF	3.6×10^4	4.56
BF	3.0×10^4	4.48
CF	1.4×10^4	4.15
DF	2.2×10^4	4.34

AUF–DUF = Unfermented ugba samples

AF–DF = Ugba samples

ND = Not detected

TCC = Total coliform count

Table 2b. Mean coliform counts.

Sample group	Mean (log₁₀ CFU/g)	Standard deviation
Unfermented ugba	3.95	±0.00
Fermented ugba	4.38	±0.18

Mean Total Heterotrophic Bacterial Counts of Unfermented and Fermented Ugba Samples

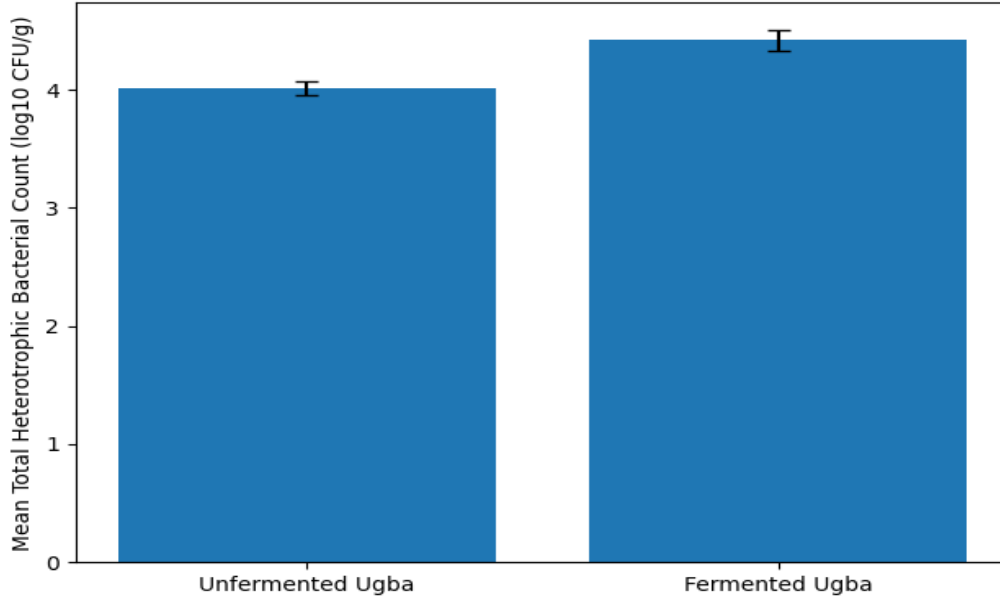


Figure 1. Mean total heterotrophic bacterial counts of unfermented and fermented Ugba samples.

Mean Total Coliform Counts of Unfermented and Fermented Ugba Samples

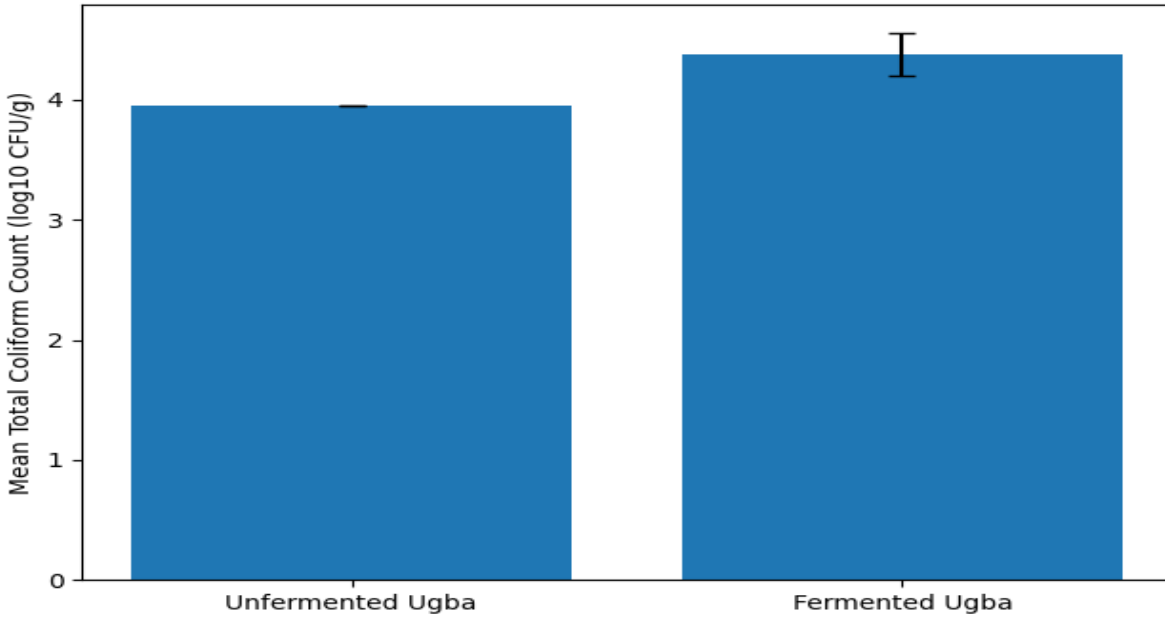


Figure 2. Mean total coliform counts of unfermented and fermented Ugba samples.

3.3. Biochemical Identification of Bacterial Isolates

The biochemical characteristics of bacterial isolates obtained from ugba samples are shown in Table 3. Based on Gram staining, motility, and biochemical reactions, the isolates were identified as *Salmonella* spp., *Klebsiella* spp., *Escherichia coli*, *Pseudomonas* spp., and *Staphylococcus aureus*.

Table 3. Biochemical identification of bacterial isolates from ugba samples.

Gram reaction	Motility	Indole	Catalase	VP	MR	Oxidase	Probable organism
–	+	–	+	–	+	–	<i>Salmonella</i> spp
–	–	–	+	+	–	–	<i>Klebsiella</i> spp
–	+	+	+	+	+	–	<i>Escherichia coli</i>
+	–	–	+	–	+	–	<i>Staphylococcus aureus</i>
–	+	–	+	–	–	+	<i>Pseudomonas</i> spp

3.4. Occurrence of Bacterial Isolates in Unfermented Ugba

The occurrence of bacterial isolates recovered from unfermented ugba samples is shown in Table 4. A total of 15 isolates were obtained. *Escherichia coli* accounted for the highest proportion (40.0%), followed by *Klebsiella* spp. (26.7%). *Salmonella* spp. and *Pseudomonas* spp. each represented 13.3%, while *Staphylococcus aureus* occurred least frequently (6.7%).

Table 4. Percentage occurrence of bacterial isolates in unfermented ugba samples.

Bacterial isolate	Frequency	Percentage (%)
<i>Salmonella</i> spp	2	13.3
<i>Klebsiella</i> spp	4	26.7
<i>Escherichia coli</i>	6	40.0
<i>Staphylococcus aureus</i>	1	6.7
<i>Pseudomonas</i> spp	2	13.3
Total	15	100

3.5. Occurrence of Bacterial Isolates in Fermented Ugba

The frequency distribution of bacterial isolates recovered from fermented ugba samples is presented in Table 5. A total of 30 bacterial isolates were obtained. The distribution pattern was similar to that observed in the unfermented samples. *Escherichia coli* remained the most frequently isolated organism (40.0%), followed by *Klebsiella spp.* (26.7%).

Table 5. Percentage occurrence of bacterial isolates in fermented ugba samples.

Bacterial isolate	Frequency	Percentage (%)
Salmonella spp	4	13.3
Klebsiella spp	8	26.7
<i>Escherichia coli</i>	12	40.0
<i>Staphylococcus aureus</i>	2	6.7
Pseudomonas spp	4	13.3
Total	30	100

4. DISCUSSION

The present study investigated the bacteriological characteristics of fermented and unfermented Ugba samples in order to understand the microbial changes associated with the fermentation process. The findings revealed that fermented Ugba samples exhibited higher total heterotrophic bacterial counts than the unfermented samples. This observation reflects the typical microbial dynamics of spontaneous fermentation systems, where microorganisms proliferate as they metabolize nutrients released during substrate degradation. Fermentation environments often favor microbial growth because enzymatic breakdown of macromolecules generates simpler compounds that support microbial proliferation. Similar increases in microbial populations during the fermentation of African oil bean seeds have been reported in earlier studies, where microbial counts increased progressively as fermentation progressed (Odunfa and Oyewole, 1998; Isu and Ofuya, 2000).

The higher bacterial load observed in fermented Ugba samples in this study is also consistent with the microbial succession commonly reported in alkaline fermentation of seed condiments. Fermentation of African oil bean seeds is typically dominated by *Bacillus* species, particularly *Bacillus subtilis*, which possess strong proteolytic capabilities. These organisms produce extracellular enzymes that hydrolyze complex proteins into peptides and amino acids, thereby contributing to the characteristic flavor and improved digestibility associated with fermented Ugba. Previous investigations have demonstrated that the proteolytic activity of *Bacillus* species plays a critical role in the biochemical transformation of African oil bean seeds during fermentation (Ouoba *et al.*, 2002). Similarly, studies on fermented seed condiments across West Africa have confirmed that the fermentation of Ugba and related products is largely driven by proteolytic bacteria that promote protein hydrolysis and nutrient release within the fermenting substrate (Parkouda *et al.*, 2009).

The increase in bacterial counts observed during fermentation in this study is also comparable to findings reported for other African alkaline fermented condiments such as *iru*, *daddawa*, and *soumbala*, where bacterial populations increase as fermentation progresses due to microbial enzymatic activities and metabolic interactions within the fermenting substrate (Omafuvbe *et al.*, 2004; Parkouda *et al.*, 2009). In such fermentation systems, microbial growth is often enhanced by the accumulation of peptides, amino acids, and other nitrogenous compounds produced through enzymatic degradation of proteins, which further supports microbial proliferation.

Despite the beneficial biochemical transformations associated with fermentation, the presence of coliform bacteria detected in some Ugba samples suggests possible contamination during processing or handling. Coliform organisms are widely recognized as indicators of sanitary quality in foods, and their occurrence often reflects inadequate hygienic practices during preparation or distribution. Traditional Ugba production typically involves manual slicing of cotyledons, repeated washing, and fermentation in open containers under ambient conditions. These processing steps may expose the product to environmental contaminants, particularly when hygienic conditions are not strictly maintained. Similar observations have been reported in studies of traditional fermented foods where microbial contamination occurred due to poor sanitary practices during production and handling (Adams and Moss, 2008; Nout and Aidoo, 2010).

Interestingly, the present study observed higher coliform counts in fermented Ugba samples compared with the unfermented samples. This finding contrasts slightly with the report of Isu and Ofuya (2000), who observed a reduction in coliform populations during controlled fermentation of African oil bean seeds due to microbial competition and environmental changes within the fermenting system. The higher coliform counts observed in fermented samples in the present study may therefore reflect contamination introduced during traditional processing practices such as handling, washing, and exposure to environmental microorganisms during fermentation.

The bacterial isolates identified in the present study included *Escherichia coli*, *Klebsiella* spp., *Salmonella* spp., *Pseudomonas* spp., and *Staphylococcus aureus*. These organisms are commonly associated with environmental contamination and have been reported in various fermented foods produced under traditional conditions. Previous studies on the microbiology of fermented African oil bean seeds have documented the occurrence of diverse bacterial populations including *Bacillus*, *Staphylococcus*, *Micrococcus*, *Escherichia*, and *Pseudomonas* species during different stages of fermentation (Azokpota, 2015; Omafuvbe *et al.*, 2004). Similarly, investigations into the microbial succession during Ugba fermentation have demonstrated that the microbial composition of the product can vary depending on environmental conditions, processing techniques, and the microbial flora associated with the raw materials (Ogueke *et al.*, 2010; Eze *et al.*, 2014).

Among the isolates recovered in the present study, *Escherichia coli* showed the highest frequency of occurrence in both fermented and unfermented samples. The presence of *E. coli* in food products is commonly regarded as an indicator of fecal contamination or poor hygienic handling during food processing. Similarly, the detection of *Staphylococcus aureus* may be attributed to contamination from food handlers, as this organism is frequently associated with human skin and mucous membranes. According to Adams and Moss (2008) and Ray and Bhunia (2014), contamination by organisms such as *E. coli* and *Staphylococcus aureus* in food products often results from inadequate sanitation, contaminated water sources, or poor personal hygiene during food preparation.

The detection of *Salmonella* species in the samples is particularly important from a public health perspective because these organisms are well-known foodborne pathogens capable of causing gastrointestinal infections and food poisoning. Similarly, the presence of *Pseudomonas* species may reflect environmental contamination, as these organisms are widely distributed in soil, water, and food processing environments. The occurrence of these organisms highlights potential microbiological risks associated with the consumption of Ugba produced under uncontrolled traditional conditions.

Although fermentation is generally recognized as a natural preservation method that enhances food safety through microbial competition and the production of antimicrobial metabolites, contamination introduced during processing may still persist if sanitary practices are inadequate. Studies on fermented foods have consistently emphasized that the microbiological safety of fermented products depends not only on the fermentation process itself but also on the hygienic conditions maintained during production, handling, and storage (Steinkraus, 2004; Adams and Moss, 2008).

Furthermore, the presence of similar bacterial species in both fermented and unfermented Ugba samples suggests that some microorganisms may originate from the raw African oil bean seeds or from environmental sources during processing. The microbial composition of fermented foods is influenced by several factors including raw material quality, fermentation conditions, microbial succession, environmental microorganisms, and handling practices during production. Recent studies employing molecular techniques have also revealed that African fermented foods harbor complex microbial communities that influence both the safety and nutritional characteristics of these traditional foods (Obafemi *et al.*, 2022).

Overall, the findings of the present study indicate that fermentation leads to increased microbial populations in Ugba while the detection of potential pathogenic bacteria highlights the importance of maintaining adequate hygiene during traditional food processing. Improving sanitary practices during Ugba production, including proper handling, the use of clean water, and the adoption of controlled fermentation techniques, may significantly reduce microbial contamination and enhance the microbiological safety and quality of this widely consumed traditional fermented condiment.

5. CONCLUSION

This study provided a comparative bacteriological evaluation of fermented and unfermented Ugba produced from African oil bean seeds (*Pentaclethra macrophylla*). The findings demonstrated that fermentation significantly increased the total heterotrophic bacterial population of the product, reflecting the metabolic activities of microorganisms involved in the fermentation process. The results support previous reports that spontaneous fermentation of African oil bean seeds encourages microbial proliferation, particularly proteolytic bacteria that contribute to the biochemical transformation of the substrate and the development of the characteristic flavor of Ugba.

However, the detection of coliform bacteria and potential pathogenic organisms such as *Escherichia coli*, *Salmonella* spp., *Klebsiella* spp., *Pseudomonas* spp., and *Staphylococcus aureus* highlights possible contamination associated with traditional processing and handling practices. The presence of these microorganisms suggests that although fermentation contributes to food transformation and preservation, inadequate sanitary conditions during production may compromise the microbiological safety of the final product.

The occurrence of similar bacterial species in both fermented and unfermented samples further indicates that some microorganisms may originate from raw materials or environmental sources during processing. Consequently, the microbiological quality of Ugba depends not only on the fermentation process but also on the hygiene standards maintained throughout production and distribution.

Overall, the findings emphasize the need for improved hygienic practices and controlled fermentation methods in Ugba production. Adoption of better handling procedures, use of clean water, and implementation of standardized fermentation techniques may significantly reduce microbial contamination while preserving the nutritional and cultural value of this traditional fermented food.

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