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BACTERIOLOGICAL SUCCESSION DURING THE FERMENTATION OF OIL BEAN PURCHASED FROM EKEONUNWA MARKET IN OWERRI MUNICIPAL, IMO STATE

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ABSTRACT

This study investigates the bacteriological succession during the fermentation of oil bean (Ugba) purchased from Ekeonunwa market, Owerri Municipal, Imo State, Nigeria. The aim was to monitor the microbial dynamics and identify the key bacteria involved in the fermentation process. Microbial populations, including Total Aerobic Plate Count (TAPC), Total Coliform Count (TCC), and Total Lactic Acid Bacteria Count (TLABC), were enumerated at 24, 48, and 72 hours of fermentation. Results showed a significant increase in TAPC (from 3.3 x 10^6 CFU/ml at 24 hours to 8.9 x 10^8 CFU/ml at 72 hours) and TLABC (from 3.1 x 10^6 CFU/ml at 24 hours to 7.5 x 10^8 CFU/ml at 72 hours), reflecting the growth of beneficial bacteria such as *Lactobacillus spp.* and *Bacillus spp.*. Conversely, the TCC decreased from 3.4 x 10^4 CFU/ml to 1.1 x 10^3 CFU/ml, indicating a reduction in non-beneficial or potentially pathogenic microorganisms. The study also identified bacterial species, including *Lactobacillus spp.*, *Bacillus spp.*, *Micrococcus spp.*, *Staphylococcus aureus*, and *Klebsiella spp.*, based on their colony morphology, microscopic features, and biochemical profiles. The microbial succession showed that *Lactobacillus spp.* and *Bacillus spp.* persisted throughout the fermentation period, while *Micrococcus spp.*, *Streptococcus spp.*, and *Proteus spp.* were more transient, with a decline by 72 hours. This succession highlights the dominance of beneficial microorganisms in the fermentation process, which is crucial for ensuring food safety, flavor development, and the microbiological stability of the final product. These findings emphasize the importance of monitoring microbial dynamics during fermentation to optimize conditions for producing high-quality and safe fermented products.

Keywords: Fermentation, oil bean, bacterial succession, *Lactobacillus spp.*, *Bacillus spp.*, microbial dynamics, food safety, Ugba

INTRODUCTION

Oil bean (Ugba or Ukpaka as it is called in Igbo language), popularly called African salad, is a ready-to-eat food, which is produced by the fermentation of African oil bean seeds (*Pentaclethra macrophylla*).

Ugba, a traditional Nigerian fermented food made from African oil bean seeds (*Pentaclethra macrophylla*), is a key dietary component in many regions of Nigeria, valued for its rich protein content and unique flavor profile. However, the fermentation process, which is primarily spontaneous and relies on the activity of naturally occurring microorganisms, is poorly understood, particularly in terms of the bacteriological succession that occurs during fermentation. The oil bean (Oil bean) seeds contain 4-17% carbohydrate, 44-47% oil which has been rich in Oleic acid (Nwokedi 2017 ; Odoemela 2005) and linoleic acid (Onwuliri *et al*., 2004)

Also , they have been found to contain 36.2-43.89% crude protein which contains the 20 essential amino acids. However , the sulphur containing amino acid content is much lower than those found in other plant proteins (Mbadiwe, 2017; Mba *et al*.,2017; Odoemelam, 2005). The high content of other essential amino acids makes the seed a potential source of protein.

Oil bean is a proteinaceous delicacy consumed by millions of people in the South-Eastern zone of Nigeria. The fermented product is rich in fats, protein and carbohydrate (Oboh *et al,* 2004). During the fermentation process, *Bacillus subtilis* plays significant roles in modifying the substrate biochemically, nutritionally and organoleptically. Although the predominant species responsible for Oil bean fermentation is *Bacillus subtilis*, other species like *B. pumilus, B. megaterium, B. lichenformis* have also been found. Oil bean production is locally done through a mixed wild bacteria fermentation of the sliced, boiled and soaked African oil bean seeds.

Previous studies have shown that the microbial population during Ugba fermentation is dynamic, with different bacterial species dominating at various stages of fermentation. This succession is influenced by factors such as temperature, pH, and the availability of nutrients. For example, early stages of fermentation are often dominated by species such as *Bacillus* spp., which play a crucial role in breaking down complex proteins into peptides and amino acids, contributing to the flavor

and texture of the final product (Njoku *et al*., 2019). As fermentation progresses, other microorganisms, including lactic acid bacteria, become more prevalent, contributing to the acidification of the product and enhancing its safety by inhibiting pathogenic microorganisms (Oguntoyinbo & Dodd, 2010).

Moreover, understanding the bacteriological succession during Ugba fermentation could have broader implications for food safety and public health. Given the spontaneous nature of the fermentation process and the potential for contamination, it is essential to identify and control harmful microorganisms that may proliferate during fermentation (Enujiugha, 2009). Research in this area could lead to the development of improved fermentation protocols, including the use of starter cultures that enhance desirable microbial activity while suppressing undesirable or harmful bacteria.

In conclusion, studying the bacteriological succession during Ugba fermentation is justified by the need to improve the quality, safety, and scalability of this important traditional food. Such research has the potential to contribute to food security and public health while preserving cultural heritage through the continued production

Fig 1.

(A) African oil bean seed, (B) Dehulled seeds of African oil bean, (C) Processed slices of the African oil bean cotyledon (Okorie and Olasupo, 2013).

MATERIALS AND METHODS

SAMPLE COLLECTION AND PREPARATION

A total of 4 packaged freshly prepared Oil bean samples were obtained from Ekeonunwa Market in Owerri, Imo state .10g each of the samples were marshed with sterile blender into 90 mL of sterile 0.1% peptone water as diluent to get homogenized slurry stock culture. Serial dilution was done for each sample to obtain 6 dilutions (10^{-1}-10^{-6}) by diluting 1 in 9 mls of sterile peptone water, first from stock culture, then from subsequent dilutions. This was repeated for 2 nd, 3rd and 4th day of fermentation.

ENUMERATION OF ORGANISMS

Bacteria count: 0.1ml each of the dilution was inoculated using pour plate technique for total aerobic plate count on Nutrient agar and incubated at 37 ^{0}C for 24hrs. Plates showing between 30 and 300 colonies were counted using the digital illuminated colony counter (Cheesbrough *et al.,* 2011). Colony counts were expressed as colony forming units per gram of sample. All counts were done in triplicate and average values were reported.

Coliform Count:

Iml of the inoculum is inoculated into MacConkey agar at 45^{0}C by pour plate method. The plate is swirled to mix and solidify. Incubate at 35-37oC for 18 to 24hrs. Dark red colonies having an estimated diameter of 5mm or more are counted (Cheesbrough *et al.,* 2011).

MICROBIAL ANALYSIS

Specimens are inoculated into the freshly prepared agar using the streaking method on freshly prepared MacConkey Agar. After streaking, the media was incubated at 37^{0}C for 24 hours

Sub-Culturing

The colonies from the media after counting were purified by sub-culturing them in fresh nutrient agar plates. After purification, the isolates were maintained using nutrient agar slant.

IDENTIFICATION OF ISOLATES.

Isolates were sub-cultured using streak plate technique to obtain pure cultures. Presumptive isolates were identified by observing their morphology on the agar plates. Gram staining and biochemical tests were carried out (Cheesbrough *et al.,* 2011).

Identification of the isolates was done by major biochemical tests, for example- Triple Sugar Iron (TSI), Motility Indole Urease (MIU), Methyl-Red (MR), Voges-Proskauer (VP) and Citrate Utilization were performed following the standard methods.

RESULT

The table shows the progression of microbial populations during the fermentation of oil bean over 24, 48, and 72 hours. The total aerobic plate count (TAPC) increased significantly from 3.3 x 10^6 CFU/ml at 24 hours to 8.9 x 10^8 CFU/ml at 72 hours, indicating the proliferation of aerobic bacteria. Similarly, the total lactic acid bacteria count (TLABC) increased from 3.1 x 10^6 CFU/ml at 24 hours to 7.5 x 10^8 CFU/ml at 72 hours, reflecting the active involvement of lactic acid bacteria in the fermentation process. The TCC dropped from 3.4 x 10^4 CFU/ml to 1.1 x 10^3 CFU/ml. This trend indicates a decline in non-beneficial or potentially pathogenic bacteria, favoring beneficial ones as fermentation progressed.

This table details the morphological, microscopic, and biochemical characteristics of bacteria isolated during fermentation. Beneficial bacteria such as *Bacillus spp.*, *Lactobacillus spp.*, and *Micrococcus spp.* show consistent acid production (indicated by "A") across various carbohydrates, suggesting their metabolic adaptability and significant roles in fermentation. *Lactobacillus spp.*, identified as long slender rods with convex, opaque colonies, is particularly important for acid production and flavor enhancement during the process. *Klebsiella spp.* and *Staphylococcus aureus* also exhibited acid production, although *Staphylococcus aureus* lacked activity on glycerol. These bacteria contribute to the biochemical transformations that occur during fermentation.

Table 1. Enumeration of bacteria succession during the fermentation of Oil bean purchased from Ekeonunwa market, Owerri. (Cfu/ml)

Fermentation Time (h)	TAPC	TCC	TLABC
24	3.3×10^6	3.4×10^4	3.1×10^6
48	4.7×10^7	2.1×10^3	4.4×10^7
72	8.9×10^8	1.1×10^3	7.5×10^8

Key:

- **TAPC**: Total Aerobic Plate Count
- **TCC**: Total Coliform Count
- **SSC**: Sulfite-Reducing Clostridia Count
- **TLABC**: Total Lactic Acid Bacteria Count

Table 2. Identification of Bacteria Isolates

Colony Morphology	Microscopy	Mannose	Xylose	Mannitol	Sucrose	Maltose	Glycerol	Probable Organisms
Opaque, flat, irregular pinhead	Long/short rods	A	A	A	A	A	A	*Bacillus spp.*
Opaque, golden yellow, spherical	Irregular cocci in clusters	A	A	A	A	A	-	*Staphylococcus aureus*

Pigmented in shades of yellows	Cocci in pairs/tetrads	ND	A	A	A	-	AG	*Micrococcus spp.*
Raised, convex, smooth, opaque	Long slender rods	+	A	A	A	A	-	*Lactobacillus spp.*
Pink, circular, translucent, entire	Single rods	A	A	A	A	A	-	*Klebsiella spp.*

Key:

- **ND**: Not Done
- **A**: Acid Production
- **AG**: Acid and Gas Production
- +: Positive
- -: Negative

Table 3: Bacterial Succession During Ugba Production

Period of Fermentation (h)	*Lactobacillus spp.*	*Bacillus spp.*	*Micrococcus spp.*	*Streptococcus spp.*	*Proteus spp.*
24	+	+	+	+	+
48	+	+	+	+	+
72	+	+	-	-	-

This table tracks the presence of five isolated bacteria at 24, 48, and 72 hours of fermentation. *Lactobacillus spp.* and *Bacillus spp.* persisted throughout the entire fermentation process, indicating their dominance and essential role in maintaining the fermentation environment. *Micrococcus spp.*, *Streptococcus spp.*, and *Proteus spp.* were detected up to 48 hours, but their absence by 72 hours suggests their metabolic activity peaked earlier, after which other dominant bacteria outcompeted them. This dynamic succession illustrates the natural progression of microbial communities during fermentation, where beneficial bacteria establish dominance while less-adapted or competitive species decline.

Discussion

The significant increase in the TAPC and TLABC observed during fermentation aligns with the findings of Nout and Aidoo (2006), who highlighted the proliferation of lactic acid bacteria (LAB) in the fermentation of legumes and other plant-based foods. *Lactobacillus spp.*, in particular, has been well-documented as a key contributor to the acidification process during fermentation, which is essential for flavor development and microbial safety (Fasogbon *et al*., 2020). Similarly, Gänzle and Follador (2012) emphasized that the proliferation of LAB results in the production of lactic acid, thereby lowering the pH and creating an environment that favors the growth of beneficial microorganisms, while inhibiting harmful pathogens.

In this study, the increase in TLABC from 3.1×10^6 CFU/ml at 24 hours to 7.5×10^8 CFU/ml at 72 hours indicates the active role of LAB in the fermentation process. This finding is in agreement with that of Ogunbanwo *et al*. (2018), who reported similar growth patterns of LAB in the fermentation of plant-based products, where they contributed to both the preservation and development of the product's sensory qualities.

The reduction in the total coliform count (TCC) from 3.4×10^4 CFU/ml at 24 hours to 1.1×10^3 CFU/ml at 72 hours suggests an effective fermentation process that reduces the presence of potentially pathogenic microorganisms. Coliform bacteria, often used as indicators of fecal contamination, are commonly found in raw foods but are typically outcompeted by beneficial bacteria during proper fermentation (Nout, 2009). This reduction in coliforms is crucial for food safety, as coliforms are known to be associated with gastrointestinal illnesses (Oloketuyi *et al*., 2015). This finding supports the observation of Oloketuyi *et al*. (2015), who showed that the

growth of LAB during fermentation inhibits the proliferation of harmful bacteria, improving the microbiological safety of the product.

The bacterial isolates identified in this study, including *Bacillus spp.*, *Lactobacillus spp.*, and *Micrococcus spp.*, are consistent with those identified in other fermentation studies. *Bacillus spp.*, for example, has been recognized for its ability to produce proteases and amylases, which help in breaking down proteins and starches during fermentation, contributing to flavor enhancement and texture modification (Fasogbon *et al*., 2020). Similarly, *Lactobacillus spp.*, which plays a dominant role in lactic acid production, is a well-known microorganism responsible for the acidification of fermented foods, which improves preservation and enhances flavor (Gänzle and Follador, 2012).

The biochemical profiles of these bacteria, such as their ability to produce acid in the presence of various carbohydrates, further supports their roles in fermentation. The presence of *Klebsiella spp.* and *Staphylococcus aureus*, while not necessarily ideal for food safety, has been noted in similar studies (Fasogbon *et al*., 2020), highlighting the complex microbial communities that can exist in fermentation environments. However, *Staphylococcus aureus* is known to produce toxins that could pose a health risk if fermentation conditions are not properly controlled (Kim *et al*., 2015).

The bacterial succession observed in this study, with the persistence of *Lactobacillus spp.* and *Bacillus spp.* throughout fermentation, and the decline of *Micrococcus spp.*, *Streptococcus spp.*, and *Proteus spp.* after 48 hours, is consistent with the findings of other researchers studying microbial succession during fermentation. Nout (2009) and Nout and Aidoo (2006) described how the initial stages of fermentation are often characterized by a diverse population of microorganisms, but as fermentation progresses, LAB and other acid-producing bacteria become dominant, which results in the displacement of less-adapted or competitive species.

The dynamic microbial community observed in this study is similar to the observations of Ogunbanwo *et al*. (2018), who noted that *Lactobacillus spp.* and *Bacillus spp.* are dominant throughout the fermentation of various plant-based foods. Their persistence is crucial for the fermentation process, as they contribute to acid production and flavor development, while also inhibiting the growth of undesirable microorganisms. The absence of *Streptococcus spp.* and *Proteus spp.* by 72 hours suggests that these bacteria were outcompeted by the more acid-tolerant LAB, supporting the idea of a natural progression toward the dominance of beneficial microorganisms as fermentation continues (Ogbonna *et al*., 2013).

The results of this study emphasize the importance of monitoring microbial dynamics during fermentation to ensure both the safety and quality of the final product. Proper fermentation conditions, including the control of temperature, pH, and time, are crucial for promoting the growth of beneficial microorganisms such as *Lactobacillus spp.* and *Bacillus spp.*, while inhibiting the growth of potentially harmful bacteria (Doulgeraki *et al*., 2012). The decline in coliform counts and the increasing dominance of LAB in this study are indicative of a well-managed fermentation process, which is essential for producing safe and high-quality fermented products.

This study provides a detailed account of the bacteriological succession during the fermentation of oil bean and Ugba, confirming the crucial roles of *Lactobacillus spp.* and *Bacillus spp.* in the fermentation process. The increase in lactic acid bacteria and the reduction of coliforms demonstrate the safety and quality improvements achieved through proper fermentation. These findings are consistent with existing literature on the microbial dynamics of fermentation and highlight the importance of managing fermentation conditions to ensure high-quality and microbiologically safe fermented products.

Conclusion

The study confirms that *Lactobacillus spp.* and *Bacillus spp.* are essential in the fermentation process of oil bean and Ugba, ensuring the quality, safety, and desired characteristics of the final products. The reduction of potentially harmful bacteria like coliforms further reinforces the safety of fermented products. Monitoring microbial succession during fermentation is crucial for producing high-quality, safe fermented foods.

Recommendations

Routine microbial enumeration should be performed to ensure that fermentation remains under favorable conditions, promoting the growth of lactic acid bacteria and suppressing pathogens. Meanwhile, introducing controlled starter cultures of beneficial microorganisms, such as *Lactobacillus spp.*, could further enhance the fermentation process and ensure consistency in product quality. Also ensuring proper hygiene practices during the fermentation process is important to reduce the risk of contamination by undesirable microorganisms, including pathogenic bacteria like *Staphylococcus aureus* and *Klebsiella spp.*

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