Identification of Volatile compounds from Pure Culture fermentation of African Oil Bean Seed using Lactobacillus fermentum and Bacillus subtilis

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Abstract

This study investigates the fermentation process of African oil bean seed (*Pentaclethra macrophylla*) into *Ugba*, focusing on the roles of *Bacillus subtilis and Lactobacillus fermentum* in flavour development. Fermentation is a traditional method that enhances food safety and quality, and *Ugba* is a culturally important condiment in Southeast Nigeria. The research employed a systematic approach, the interaction between boiling and soaking durations on microbial activity and the resultant compounds that are volatile produced during fermentation. Samples of the seeds of African oil beans were subjected to different boiling and soaking times before fermentation with both single and co-cultures of the selected microorganisms. The volatile components were examined using a gas chromatography-mass spectrometry; i.e. (GC-MS), revealing 24 distinct compounds, with co-culture fermentation yielding a more diverse profile compared to single cultures. Notably, 2-nonen-1-ol was identified in co-culture fermentation, indicating enhanced flavour complexity. In naturally fermented *Ugba*, 14 volatile compounds were produced with methyl stearate, n-hexadecanoic acid, methyl ester, and oleic acid being more consistent and predominant. However, *Bacillus subtilis* pure culture fermentation produced 27 volatile compounds with methyl stearate, methyl ester and n-hexadecanoic acids being more prominent while *Lactobacillus fermentum* fermentation produced 34 compounds with hexadecanoic acids, methyl esters, and 9- octadecanoic acids being more prominent. Co-culture fermentation produced 24 volatile compounds including 2-nonen-1-ol which was not prominent as found in single/pure form fermentation. The main flavour components may be Methyl esters of different long-chain fatty acids depending on their stability and consistency in both single-culture and co-culture fermentation. This research not only contributes to the understanding of *Ugba* fermentation but also presents opportunities for standardizing production processes to achieve consistent flavour profiles. The insights obtained from this study have potential applications in the food industry, particularly in enhancing the sensory attributes of fermented products and expanding their culinary uses.

Introduction

Fermentation is a traditional method utilized globally for the enhancement of food quality, safety, and flavour. In many African cultures, oil seed fermentation, including that of African oil bean seed (*Pentacletha macrophylla*), holds significant cultural and nutritional value as a renowned condiment referred to as *Ugba* according to the Igbo extract of Southeast Nigeria. The production processes and fermentation times differ among various producers and the outcome results in a non-uniform product. On the other hand, the fundamental and traditional production approach entails the boiling of the seed for several hours, the seed coat is removed, the cotyledons are sliced, soaked and washed in water for a period and the final product is wrapped in banana leaves for fermentation (Obeta, 2008).

African oil bean process of fermentation is an alkaline mixed culture procedure primarily driven by the metabolic activities of a range of microorganisms, which can profoundly influence the final product's characteristics though the production of various volatile compounds (Reginald and Bassey, 2017). Most of the research on fermented foods in Africa has been on isolating and identifying beneficial bacteria

participating during fermentation. Among the diverse microbial communities that participate in the fermentation of *Ugba*, *Bacillus* and *Lactobacillus* spp. are the prominent microorganisms actively responsible for fermenting *Ugba* (Okorie & Olasupo, 2013; Odunfa, 1981; Obeta, 2008; Isu & Ofuya, 2000; Obeta, 1983). *Bacillus* spp. is known for its proteolytic activity, which can lead to generation of peptides and amino acids which further transform into a wide range of volatile flavour compounds (Ahaotu, et al., 2013). Conversely, *Lactobacillus* spp. contributes to the acidification of the fermenting substrate, which inhibits the growth of spoilage organisms and also influences the formation of volatile organic acids and alcohols (Nwokeleme and Ugwuanyi, 2015). However, other bacteria such as *Micrococcus Escherichia coli*, *Enterococcus*, *Alcaligenes, Pseudomonas, Streptococcus, Staphylococcus, Corynebacterium*, and *Proteus* have also been observed to be involved in the natural fermentation process of this product (Sanni, Onilude & Fadahunsi, 2002). Importantly, fungi and species of yeast are not implicated in the fermentation of *Ugba* (Njoku, Ogbulie & Nnubia, 1990). Lactic acid fermentation is well-known as a common method of food preservation and shelf-life improvement. For example, in the sub-region of West Africa, tubers and cereals are processed into a range of cuisines while in other regions, milk is converted to yoghurt and cheese. This method has proven quite efficient in maintaining the safety and quality of food, particularly in homes, where a lot of conventional meals are prepared (Steinkraus, 1983).

The indicator and quality of index of *Ugb*a are based on its flavour (Ogueke, Nwosu, Owuamanam & Iwouno, 2010) which plays an essential role in (product) acceptance. Interestingly, this has been the first quality attribute *Ugba* consumers perceive. Even though a wide range of bacteria are involved in the fermentation process of *Pentaclethrra macrophylla*, only spp. of *Bacillus*.is required to produce characteristic flavour (Beaumont, 2002). *Lactobacillus* spp. on the other hand, has also been reported to have a significant contribution to the quality characteristic of *Ugba*, indicating its probiotic potential (Anosike et al., 2022). Despite the distinct flavour and other organoleptic qualities of African oil bean seeds that have undergone fermentation, there are variations (in terms of flavour intensity) among different *Ugba* products because of microbial diversity, fermentation conditions, substrate composition, and processing method/treatment (Kabuo, Uzuegbu, Ubbaonu & Onyeka, 2007). Just as several studies have conducted the microbiological characterization of African oil bean seeds fermentation (Njoku & Okemadu, 1989), there is limited research on how pretreatment techniques affect the volatile flavour compounds identified during *Ugba* fermentation with pure culture microorganisms. Against this background, the aim of this study is to identify the volatile aroma compounds of *Ugba* subjected to some pretreatments and pure culture fermentation. A good understanding of these factors can help in standardizing fermentation processes and achieving consistent flavour profiles in African oil bean seeds after fermentation.

Materials and Methods

Collection of Sample

The *Ugba* seeds were sourced from dealers in Relief market, Owerri, Imo State, Nigeria. After visual inspection, the defective *Ugba* seeds were removed. Every chemical and reagent used in this study was

of analytical quality.

Preparation of sample

Traditional Ugba Production

The preparation of *Ugba* was carried out according to Njoku et al. (1990). The *Ugba* seeds were boiled for 6 h and the hard coat dehulled. The resulting cotyledons were cooled, washed, and sliced. The cotyledons were washed repeatedly and allowed to boil again for a duration of 2 h, cooled and then soaked in water for 12 .The cotyledons were washed and kept in a basket to drain for 1 h and put in wraps in portions of 50g of slices using *Mallotus oppositifolius* leaf and incubated for 72 h at 35 ± 2 ^{o}C. The product obtained after fermentation is called *Ugba* and the flow diagram for the production is shown in Fig. 1.

Pure culture Ugba production

The preparation of *Ugba* was done according to method of Njoku, et al. (1990). All samples were treated for a total of 12 h period. The raw seeds were boiled for 2, 4, and 6 h and subsequently soaked for 10, 8, and 6 h respectively. These samples were labelled A, B, and C respectively .To reduce bitterness, the slices were washed several times in water after soaking and kept to drain. This was sterilized / autoclaved in conical flask and allowed to cool. Starter cultures were inoculated singly and in combination. This was allowed to ferment for three days.

Microbial isolates characterization and identification

The isolates' colonies were inoculated on nutrient agar medium using streak plate method and cultured for 48 hours at room temperature anaerobically. Pure cultures obtained through isolation were subcultured and stored at 4°C in nutrient agar slants. Microbial isolates were identified using conventional protocols and cultural (colonial), microscopy, and biochemical methods (Cheesbrough, 2000). Standard guidelines for identification of bacteria were used to confirm the identities of the isolates.

The microorganisms were microscopically characterized using the gram staining technique, as reported by Cheesbrough (2000). Cheesbrough (2000) procedures were used to further describe the isolates' identities biochemically.

P**reparation of pure culture isolates as starter cultures**

Bacillus subtilis and *Lactobacillus fermentum* isolates from naturally fermented *'Ugba'* were inoculated separately into 20 mL of sterile water with 0.1% peptone and incubated for 24 h. Centrifugetion was used on the cultures after incubation, and both distilled water and saline were used to repeatedly wash the

culture pellets before being distributed on freshly produced medium to determine viability, purity, and biomass of the cells

Pure culture fermentation

Using a sterile micropipette, 1 mL of the isolates suspension both individually and collectively, was aseptically inoculated into separate flasks and thoroughly mixed with the *Ugba* sample. The fermentation process continued for 72 hours with periodic flask agitation. To verify the process's purity, one gramme (1g) of the *Ugba* fermenting slices was collected every 24 hours and also at the conclusion of the fermentation process and stirred in nine milliliter (9 mL) peptone water that is sterile with 0.1% concentration. Smear from this *Ugba* sample was used for isolation and characterization using streak method on a nutrient agar plate.

Quality evaluation of Ugba products

Sample preparation for Analysis of volatile flavour compounds

This was carried out using Nwokeleme and Ugwuanyi approach (2015). *Ugba* fermented with pure isolate culture and *Ugba* fermented naturally were used to obtain samples for analysis. For 3 days, samples were taken at 12 h intervals. To extract volatile taste components, Wet milling was used to grind one gramme (1g) of the sample that was fermenting, which was then homogenized in nine mililitre (9 mL) of diethyl ether. Filteration method using filter paper was adopted to seperate the mixture, and Millipore method of filtration was used to separate particulate debris from the filtrate using a membrane filter of a pore size of 0.46 micro meter. Filtrate obtained was sealed, placed in little ampoules, and kept until analysis was needed.

Gas Chromatography–Mass Spectrometry Analysis (GC-MS)

Analysis was done using GC-MS in electronimpact (EI) mode with a 70 eV ionization voltage. One micro litre (1 µL) of sample was injected, and analysis was performed in split injection mode. The GC column oven temperature was 60°C, and the injector temperature was 250°C, with a flow rate of 53.3 mL/min at a temperature of 250°C at the interface. Helium was the carrier gas, and the pressure was 56.2 kPa. The oven temperature was 280°C for five minutes after being kept at 60°C for two minutes and then increased to 180°C for three minutes. The solvents cut time regime was 2.50 minutes, and the source of ion's temperature was 200 ^{o}C. By contrasting the mass spectra, retention indices, and chomatographic retention durations of the fermented African oil bean seeds using those of the real materials and information in the NIST0S Mass Spectral Library, the peak identification was verified.

Results and Discussion

A total number of seven bacterial genera were isolated from the naturally fermented *Ugba*. Characterization of these organisms confirmed them to be *Serratia* sp, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Bacillus megaterium*, *Enterococcus faecalis, Bacillus subtillus* and *Lactobcillus fermentum*. (Table 1).

Microorganisms that were isolated from African oil bean seed (*Ugba*) after fermentation are the same as those earlier isolated by other researchers (Isu & Njoku, 1997 ,Obeta 1983). Both indigenous and contaminating bacteria which were isolated from naturally fermented *Ugba*. *Lactobacillus* and *Bacillus* species are indigenous bacteria involved in the fermentation of *Ugba*. *Pseudomonas* and *Staphylococcus* species are proteolytic bacteria present as contaminants. This is in agreement with what was reported by Ogueke and Aririatu (2004). The erratic and unpredictable source of organism (inoculum) for *Ugba* production partially explains the large variety of microorganisms which were isolated.

The isolates morphological and physiological characteristics revealed that they were rod-shaped, gram-positive, motile, catalase-positive and spore-forming. These characteristics are typical of *Bacillus* isolates and *Bacillus* spp was the predominant of all the isolated organisms. This, however, was reported in prior results (Sanni et al., 2002; Enujiugha, 2009). The dominance of *Bacillus* may be due to their ability to degrade the rich protein content of *Ugba* and produce spores that are resistant to the heat from the long cooking hours of *Ugba* (Ahaotu et al., 2013). Other isolates were gram-negative, cocci as well as the catalase-negative, suspected to be *Micrococcus* and *Staphylococcus* spp.

Chromatograms of the volatile elements of un-inoculated fermented *Ugba* and inoculated fermented *Ugba* with *Bacillus subtilis, Lactobacillus fermentum* and combination of both isolates (co-culture) for 24 h and 72 h are shown in Table 2–5. The chromatogram graphs are shown in the appendix. The highest peak in the chromatogram of the control sample at 24 h and 72 h was observed at 6.017 mins for both periods. The highest peak in the chromatogram of sample A Isolate A at 24 h and 72 h was observed at 16.416 mins and 16.396 mins respectively. The highest peaks in the chromatogram of sample A Isolate B at 24 h and 72 h were observed at 15.988 and 16.057 mins respectively. The highest peaks in the chromatogram of sample A Isolate C at 24 h and 72 h were observed at 15.988 and 16.396 mins respectively. The greatest peaks in the chromatogram of sample B Isolate A at 24 h were observed at 17.433 min. The greatest peaks in the chromatogram of sample B isolate B at 24 h and 72 h were observed at 17.433 and 16.039 mins respectively. The highest peaks in the chromatogram of sample B Isolate C at 24 h and 72 h were observed at 17.433 and 15.042 mins respectively. The highest peaks in the chromatogram of sample C Isolate A at 24 h and 72 h were observed at 16.488 and 16.061 mins respectively. The chromatograms greatest peaks of sample C Isolate B at 24 h were observed at 16.040 mins. The chromatograms greatest peaks of sample C Isolate C at 24 h and 72 h were observed at 16.040 and 16.014 mins respectively.

The names of the volatile components of un-inoculated fermented *Ugba* and inoculated fermented *Ugba* with *Bacillus subtilis, Lactobacillus fermentum* and combination of both isolates for 24 h and 72 h are presented in Tables 2–5. 9-Octadecadienoic acid (E) and its esters were the highest volatile component

observed in Sample A Isolate A at 24 h having an overall percentage concentration of 25.924% and an average retention time of 16.416 min, while esters of 9,12-Octadecadienoic acids (Z,Z) were the greatest values recorded in Sample A Isolate A at 72h having a concentration percentage of 222.872% with a retention time of 16.341 min. In Sample A Isolate B at 24 h and 72 h, the highest volatile components observed were 9-Octadecenoic acid (Z)-, with its ester (21.197%) and 8,11-Octadecadienoic acid and its esters (24.147%) respectively. In Sample A Isolate C at 24 h and 72 h, the highest volatile components observed were 9-Octadecenoic acid (Z)-,with its ester (19.394%) including Methyl stearate (22.872%) respectively. In Sample B Isolate A at 24 h the highest volatile components observed were esters of 9,12-Octadecadienoic acids (Z,Z)- (17.816%). In Sample B Isolate B at 24h and 72h, the highest volatile components observed were Methyl 12-hydroxy-9-octadecenoate and its ester (17.816%) and 9-Octadecenoic acids (Z)- and its esters (34.326%) respectively. In Sample B Isolate C at 24 h and 72 h, the highest volatile components observed were Methyl 12-hydroxy-9-octadecenoate and its Ester (17.816%) and 9-Octadecenoic acid (Z)- and its esters (34.326%) respectively. In Sample C Isolate A at 24 h and 72 h, the highest volatile components observed were 9,12-Octadecadienoic acids (Z,Z)- with its ester (30.062%, 25.711% respectively). In Sample C Isolate B at 24h, the highest volatile components observed were 11-Octadecenoic acid and its ester (35.454%). In Sample C Isolate C at 24 h and 72 h, the highest volatile components observed were 11-Octadecenoic acid and its Ester (35.454%) and 9,12-Octadecadienoic acid (Z,Z)-, and its esters (20.851%) respectively. While in the control sample at 24 h and 72 h, the highest volatile component observed was Oleic acid (23.083%).

Among the biggest classes of volatile flavour compounds generated in natural fermentation, mixed-culture and pure culture fermentation is methyl esters of fatty acids (Nwokeleme and Ugwuanyi, 2015). It has been reported that methyl esters are genarated through the use of enzymes that participate in fermentation by the methanolysis of acyl-COA produced via fatty acid synthesis or degradation (Nwokeleme and Ugwuanyi). In this study, methyl ester was the largest observable volatile compound present in all samples including the control. As already suggested in previous reports that Methyl esters are responsible for volatile flavour compounds in fermented food products (Perestrelo et al., 2006). Reginald and Bassey (2017) have reported a high percentage concentration of 9,12-Octadecadienoic acids (Z,Z)- in seed of *Ugba*. In this study 9,12-Octadecadienoic acid (Z, Z)- was also present in all samples at 72 h except for the control sample at 72 h. However, its presence in inoculated fermented samples might be a result of the capacity of the microorganisms to start an enzymatic biochemical process leading to its synthesis.

The volatile substances that give *Ugba* its distinct smell and odour are short-chain fatty acids, which are created by microbiological activity. It has been reported that the hexadecanoic acid found in all of the study's samples had medicinal qualities. Hexadecanoic acid's capacity to regulate insulin secretion through the nervous system which also suppresses the natural appetite-suppressing signals of the body from leptin and insulin in rats fed with diet consisting of 80% carbohydrates and 20% palmitic acid is thought to be responsible for its potential to regulate body weight (Benoit et al., 2009; Reginald and Bassey, 2017). Despite indications of hexadecanoic acid's significant ability to cause metastases in cancer found in humans, (Pascual et al., 2016), the propensity of hexadecanoic acid to induce

metastases may be counteracted by the high content of 9-Octadecenoic acid (Z)-, methyl ester found in these foods that have been fermented (Reginald and Bassey, 2017). Consuming 9-Octadecenoic acid (Z)-, methyl ester (oleate) salt has shown to reduce cancer of the breast in women, according to Reginald and Bassey (2017). Hexadecanoic acid's capacity to induce metastases may be strongly inhibited by the properties of 9-Octadecenoic acid (Z)-, methyl ester which are anti-cancerous (Reginald and Bassey, 2017). The ingestion of 9-Octadecenoic acid (Z)-, methyl ester (oleic acid), which is a monounsaturated omega-9 fatty acid, has been linked to a rise in HDL and a corresponding drop in LDL This suggests that *P. macrophylla* Benth seed when fermented, could help in managing and treating hypertension. The third day of fermentation also showed a significant concentration of methyl stearate, which is consistent with the findings of Reginald and Bassey (2017), who also noted a high concentration of methyl state on the fourth day of fermentation. This chemical is a significant surfactant and softening agent due to its nonpolar chain's solubility in organic solvents and its polar head group's bifunctional capacity to attach metal cations.

An increase in fermentation time could be the cause of the fermented *Ugba* seed's increased softness and improved emulsification in palmitic oil (Reginald and Bassey, 2017). Despite the presence of *Lactobacillus fermentum* and *B. subtilis* in pure fermentation, the volatile profile of the traditional and pure culture fermentation was different. This makes sense because both type, amount, and duration of various volatile chemicals may be modulated by the mixing of other microbes present in the natural process.

Evolution of volatile substances was affected by the duration of boiling and soaking time as observed in Table 2 to Table 5 and this agrees with the report of Kabuo, et al, (2007). Boiling and soaking also modify the seeds' physical and chemical properties, affecting nutrient accessibility (Fernandez Castaneda et al., 2024). Prolonged boiling can break down complex carbohydrates and proteins, enhancing their availability to fermenting microorganisms. The breakdown of plant chemicals and the production of metabolites by the active populations of microorganisms in charge of fermentation result in synthesis of *Ugba* flavours during fermentation. Sample A, Isolate B evolved 25 volatile compounds which is the highest number of volatile compounds evolved in all the samples. This is understandable as Enujiugha, *et al*., (2004) reported that may be fermentation started very early during the soaking of the sliced beans cotyledon. This also agrees with Ogueke and Aririatu, 2004 report which suggested that boiling of *Ugba* seeds for several hours inactivates the activity of enzymes involved in *Ugba* fermentation.

Boiling impacts seed texture and nutrient availability, while soaking affects water absorption and seed softening (Kajihausa et al., 2014; Schoeninger et al., 2014). As soaking time increased to 10 h, sample A isolate B released 25 volatile compounds after 72 h of fermentation and the following volatile compounds were evolved which were not seen in other samples, namely; 10, 13 - octadecadienoic acid(Z,Z)- Methyl ester, 8, 11, octadecadienoic acid, methyl ester and 9H- purine-6 (1H) - throne, 9-(piperidine methyl) while sample A, isolate A released 12 volatile compounds which include 2(3H)-Furanone, dihydro-5-2-octen and 2(3H)-Furanone,5-hexyldihydroester. Sample A isolate C released 10 volatile compounds and only this sample in samples A released 2-Nonen-1-ol. Adequate hydration is

essential for microbial activity, as it supports the metabolic processes necessary for effective fermentation (Tapia et al., 2020). Insufficient soaking may cause incomplete fermentation, whereas excessive soaking can cause nutrient leaching, both of which may compromise the final product's quality.

Additionally, variations in boiling and soaking times influence the substrate's pH (Yarlina et al., 2023), which is critical for the growth of specific microorganisms. These pH changes can affect fermentation kinetics, the production of volatile compounds, and ultimately the aroma and flavor profile of the fermented product. As boiling time increased to 6 h, sample C, isolate A released 24 volatile compounds and only this sample released Terpinen – 4 - ol after 72 hrs of fermentation. Sample C, isolate B released 23 volatile compounds, and this sample has no unique volatile compounds released compared to other samples. Sample C, Isolate C released 18 volatile compounds with Tetradecanoic acid, 12 methyl esters as it's unique volatile compounds.

Table 1. Biochemical and sugar fermentation of bacterial isolates

CaT	OX	COL	IN	MR	VP	Cit	UP	NO_3	CHO	Suc	Lac	Maly	XL	Probable Identity of isolates
+	-	-	-	-	+	+	-	+	+	+	+	+	+	*Serratia* sp
+	+	-	-	+	-	+	-	-	+	+	-	-	+	*Pseudomonas aeruginosa*
+	-	-	-	-	+	-	+	+	+	+	+	+	-	*Staphylococcus aureus*
+	-	-	-	-	+	+	-	+	+	+	-	-	+	*Bacillus megaterium*
-	-	-	-	+	-	+	-	-	+	+	+	-	-	*Enterococcus faecalis*
+	-	-	-	-	+	+	-	+	+	-	-	+	-	*Bacillus subtilis*
-	-	-	-	+	-	+	-	-	+	+	-	-	+	*Lactobacillus* sp

Keys: CaT= catalase; OX=oxidase; COL= coagulase; IN= indole; MR= methyl red; VP= voges proskaeur, CiT= citrate, UP= urease production; NO_3= nitrate reduction; CHO= glucose; Suc= sucrose; Lac= lactose; Malt= maltose; XL= xylose.

Table 2. Volatile flavour components released and identified in pure cultures fermentation of *Ugba* with *Bacillus subtilis*

Sample	24h	72h
PAA	• Pentadecanoic acidS, 14-methyl- esterS • 9,12-Octadecadienoic acids (Z,Z)- • n-Hexadecanoic acids • Octadecanoic acids • 9-Octadecenoic acid, methyl esters • Methyl stearate • Heptadecanoic acids, 16-methyl ester • 8-Octadecenoic acids, methyl esters • 9-Octadecenoic acids • 9,12-Octadecadienoic acids (Z,Z)-methyl esters • 1,3-Cyclohexanediol-trans-1H-Tetrazole-1-ethanol, 5-amino-Tetraacetyl-d-xylonic nitrile • 2-Propenamide, N-(1-cyclohexylethy l)-2-Pyrrolidinon • Glycerol 1-palmitate • cis-9-Hexadecenal • Octadec-9-enoic acids	• dihydro-5-(2-octen 5-hexyldihydro-esters, • 2(3H)-Furanone, 2(3H)-Furanones, • Pentadecanoic acids, 14-methyl-esters • n-Hexadecanoic acids • 10,13-Octadecadienoic acids, methyl-esters • methyl esters, 11-Octadecenoic acids, • Methyl stearates • Heptadecanoic acids, 16-methyl-esters • 9,12-Octadecadienoic acids (Z,Z)- Linoelaidic acids • 6-Octadecenoic acid, (Z)- • Octadecanoic acid -1,11-Dodecadienes • cis-Vaccenic acids • 9,17-Octadecadienals, • 9,12-Octadecadien-1-ol, (Z,Z)-
PBA	• Linoelaidic acids 9,12-Octadecadienoic acids (Z,Z)-methyl ester • 9-Octadecenoic acid (Z)-, methyl ester • Methyl stearate • n-Hexadecanoics acids • cis-Vaccenic acids • 9-Octadecenoic acids • Methyl 12-hydroxy-9-octadecenoates • 1,1'-Bicyclohexyl]-2-one • 1,7-Dioxaspiro[5.5]undec-2-ene • 1,5-Di(1-piperidinyl)pentane • 1H-Imidazole-2-methanols • cis-13-Octadecenoic acids • Oleic acids	• Catechol • 4-Ethylcatechols • 1,3-Benzenediol, 4-ethyl- • 1,3-Benzenediol, 4-ethyl- • Hexadecanoic acid, methyl esters • Pentadecanoic acid, 14-methyl-, ester • n-Hexadecanoic acids • n-Hexadecanoic acids • 9-Octadecenoic acids, methyl esters • cis-13-Octadecenoic acids, methyl esters • Methyl stearate • 9,12-Octadecadienoic acid (Z,Z)- • Linoelaidic acids • 9,17-Octadecadienal, (Z)-

		• Octadecanals
PCA	• Methyl stearate • Dimethyl phthalates • 2(3H)-Furanone, dihydro-5,decan-9-ol. • gamma-Dodecalactone • Pentadecanoic acids, 14-methyl-esters • n-Hexadecanoics acids • 9,12-Octadecadienoic acids (Z,Z) –methyl esters • 9-9-Octadecenoic acids (Z)-, • 12-Octadecenoic acids, methyl esters • Hexadecanoic acid, methyl esters. • 9,12-Octadecadienoic acids (Z,Z)- lineoleic acids 9-Octadecenoic acids, (E)- lineoleic acids • 9-Oxabicyclo[6.1.0]nonane • Dodecanoic acids, 10-methyl-esters • Disparlure	• Terpinen-4-ols • Terpinen-4-ols • 3-Cyclohexen-1-ol, 4-methyl-1-(1-m ethylethyl)-, (R)- • Hexadecanoics acids, methyl esters • Pentadecanoic acids, 14-methyl-esters • 9,12-Octadecadienoics acids (Z, Z)-, methyl esters • n-Hexadecanoics acids • 6-Octadecenoic acids, methyl esters • Methyl stearate • 9,12-Octadecadienoic acidss (Z, Z)-, methyl esters • 9,12-Octadecadienoics acids (Z,Z)-, methyl esters • 9-Octadecenoic acids, methyl esters • methyl esters 9-Octadecenoic acids (Z)-, • trifluoroacetates • cis-Vaccenic acids • 9-Octadecenoic acids (Z)-, 2,3-dihy droxypropyl esters • Trans-2-Dodecen-1-ol, trifluoroacetates • Z-11-Tetradecenoic acids • Eicosanoic acid, methyl esters • Docosanoics acids, methyl esters • Methyl 20-methyl-heneicosanoates • trans-13-Octadecenoic acids • Oleic Acids • Oleyl alcohols

Table 3. The Volatile flavour components released and identified in a pure culture fermentation procrss of *Ugba* with *Lactobacillus fermentum*

Sample	24 h	72 h
PAB		
	• 9,12-Octadecadien-1-ol • Methyl stearates • n-Hexadecanoic acids • 9,12-Octadecadienoic acids (Z,Z)-methyl esters • Hexadecanoic acid, methyl esters • 9,12-Octadecadienoic acids • 9-Octadecenoic acid (Z)-, methyl esters	• 9,12-Octadecadienoic acids (Z, Z)-, methyl esters • 4-Ethylcatechol • 10,13-Octadecadienoics acids, methyl esters • Methyl stearates • 9-Octadecenoic acids, methyl esters • cis-Vaccenic acids • cis-13-Octadecenoics acids, methyl esters
	• 9-Octadecenoic acids	• 1,3-Benzenediols, 4-ethyl- • 1,3-Benzenediols, 4-ethyl- • Hexadecanoic acids, methyl esters • Pentadecanoic acids, 14-methyl, esters • n-Hexadecanoics acids • n-Hexadecanoic acids • 8,11-Octadecadienoic acids, methyl esters
		• 9,12-Octadecadienoic acids (Z,Z)- • Linoelaidic acids • 9,12-Octadecadienoic acid (Z,Z)- • cis-13-Octadecenoic acid • 9-Octadecenoic acid
		• 1,7-Dioxaspiro[5.5]undec-2-ene • 3,3'-Iminobispropylamine • 9H-Purine-6(1H)-thiones, 9-(piperidinomethyl)- • Eicosanoic acids, methyl esters • 9,17-Octadecadienals, (Z)- • Octadecanals
PBB		
	• Oleic Acids • Hexadecanoic acids, methyl esters • n-Hexadecanoic acids • 9,12-Octadecadienoic acids (Z,Z)-, methyl esters • 11-Octadecenoic acids, methyl esters	• Hexadecanoic acids, methyl esters • Methyl stearates • n-Hexadecanoics acids • 9,12-Octadecadienoic acids (Z,Z)-, methyl esters • 9,17-Octadecadienals, (Z)-

	• cis-10-Heptadecenoic acids, methyl esters • Methyl stearates • 9,12-Octadecadienoicsacids (Z,Z)-Linoelaidic acids • cis-Vaccenic acids • Methyl 12-hydroxy-9-octadecenoates • 1H-Tetrazole-1-ethanol, 5-amino-1,5-Di-1-piperidinyl pentanes • 9-Octadecenoic acid (Z)-, 2-hydroxy1 hydroxymethyl ethyl ester • Pentadecanoic acids, 14-methyl methyl esters	• 9-Octadecenoic acids (Z)-, methyl esters • 9-Octadecenoic acids (Z)-, methyl esters • 9-Octadecenoic acids (Z)-, 2,3-dihydroxypropyl esters • 9-Octadecenoic acids
PCB	• 11-Octadecenoic acids, methyl esters • Pentadecanoic acid, 14-methyl-esters • Hexadecanoic acids methyl esters • Docosanoic acids, methyl esters. • cis-13-Octadecenoic acids, methyl esters • Methyl stearates • Heptadecanoic acids, 16-methyl-, methyl esters • Hexadecanoic acids, 14-methyl esters • Eicosanoic acids, methyl esters • Octadecanoic acid, 11-methyl-esters • Methyl 20-methyl-heneicosanoates.	• Hexadecanoic acids, methyl esters • Hexadecanoic acid, methyl esters • Pentadecanoic acids, 14-methyl-methyl esters • cis-13-Octadecenoic acids, methyl esters • 9-Octadecenoic acids (Z)-methyl esters • 10-Octadecenoic acids, methyl esters • Cis-13-Octadecenoic acid, methyl esters • Methyl stearates • 9,12-Octadecadienoic acids • Linoelaidics acids • 9,12-Octadecadienoics acids • 9-Octadecenoic acids • cis-13-Octadecenoic acids • 9-Octadecenoic acids • cis-Vaccenic acids • 1,7-Dioxaspiro[5.5]undec-2-ene • 3,3'-Iminobispropylamines • Eicosanoic acids, methyl esters • 9,17-Octadecadienals • Catechols • 4-Ethylcatechol • 1,3-Benzenediol, 4-ethyl- 9-Octadecenal • 13-Octadecadien-1-ol

Table 4. Volatile flavour compounds released and identified in pure culture fermentation of *Ugba* with *Bacillus subtilis* and *Lactobacillus fermentum*

Sample	24 h	72 h
PAC	• Hexadecanoic acid, methyl ester • Oleic Acid • 12-Octadecadienoic acids (Z,Z)-methyl esters, • 9-Octadecenoic acids (Z)-, methyl esters • Methyl stearate • 9-Octadecenoic acids (Z)-, methyl esters • 10-Octadecenoic acids, methyl esters • 9-Octadecenoic acids, methyl esters • N-Hexadecanoic acids • 9,17-Octadecadienal, (Z)- • 9-Octadecenoic acids (Z)-, 2,3-dihy-droxypropyl esters	• Hexadecanoic acid, methyl esters • n-Hexadecanoic acids • 9,12-Octadecadienoic acids (Z,Z)-, methyl esters • 9-Octadecenoic acid (Z)-, methyl esters • 9-Octadecenoic acids (Z)-, methyl esters • Methyl stearates • 9-Octadecenoic acids • 9,17-Octadecadienal, (Z)- • 9-Octadecenoic acids (Z)-, 2,3-dihydroxypropyl esters • 2-Nonen-1-ol, (Z)-
PBC	• Pentadecanoic acids, 14-methyl esters • n-Hexadecanoic acids • 9,12-Octadecadienoic acids (Z,Z)-, methyl esters • cis-Vaccenic acids • 9-Octadecenoic acids (Z)-, methyl esters • Cis-10-Heptadecenoic acids, methyl ester • Methyl stearate • 9,12-Octadecadienoic acid (Z,Z)- • Oleic Acid • 1,7-Dioxaspiro[5.5]undec-2-enes • 9-Octadecenal, (Z)- cis-13-Octadecenoic acids -	• 9-Oxabicyclononanes • 9,12-Octadecadienoic acids (Z,Z)- • Pentadecanoic acid, 14-methyl-, methyl esters • Hexadecanoic acid, methyl esters • Methyl 10-trans,12-cis- octadecadinoates • Methyl stearates ◦ 9,12-Octadecadienoic acids (Z,Z)-, methyl esters • 9-Octadecenoic acids, methyl esters • 2(1H)-Benzocyclooctenone, decahydr o-4a-methyl-, trans-(-)- 2-Butenediamide, 2-methyl-, (Z)- • Hexadecanoic acids, 14-methyl esters • Eicosanoic acids, methyl esters • Methyl 20-methylheneicosanoates • Heneicosanoic acids, methyl esters • Methyl 14-methyl-eicosanoates • Tetracosanoic acids, methyl esters • 6-Octadecenoic acids trans-13-Octadecenoic acids

PCC

- 11-Octadecenoic acids, methyl esters
- Pentadecanoic acids, 14-methyl- esters
- Methyl 20-methyl-heneicosanoate
- 9,12-Octadecadienoic acid (Z, Z)-, methyl esters
- 9,12-Octadecadienoic acid, methyl esters
- Hexadecanoic acids, methyl esters
- 9-Octadecenoic acids, methyl esters,
- 9-Octadecenoic acids, methyl esters,
- 11-Octadecenoic acid, methyl ester
- Methyl stearate
- Heptadecanoic acid, 16-methyl-,ester
- Hexadecanoic acid, 14-methyl-ester
- Methyl 18-methylnonadecanoate
- Eicosanoic acid, methyl ester
- Docosanoic acid, methyl ester
- Octadecanoic acid, 11-methyl-ester
- Hexadecanoic acids, methyl esters
- Pentadecanoic acids, 14-methyl esters
- Hexadecanoic acids, methyl ester
- n-Hexadecanoic acids
- 9,12-Octadecadienoic acids (Z, Z)-, methyl esters
- 9,12-Octadecadienoic acid (Z,Z)-, methyl esters
- Methyl 10-trans,12-cis-octadecadi noates
- cis-13-Octadecenoic acids, methyl esters
- 9-Octadecenoic acid (Z)-, methyl esters
- Methyl stearates Heptadecanoic acid, 16-methyl-, methyl esters
- 9,12-Octadecadienoic acids (Z,Z)-
- Oleic acids
- Hexadecanoic acids, 14-methyl methyl esters
- Tetradecanoic acids, 12-methyl esters
- heptadecanoic acids, methyl esters
- Oleic acids
- cis-Vaccenic acids
- 9-Octadecenoic acids

Table 5: Volatile flavour Compounds released and Identified in Traditional Fermentation of *Ugba*

Control sample at 24 h	Control sample at 72 h
• Hexadecanoic acids, methyl esters • n-Hexadecanoic acids • 9,12-Octadecadienoic acids, methyl esters • 9-Octadecenoic acids, methyl esters • 10-Octadecenoic acids, methyl esters • Methyl stearate • Oleic Acids • 1-Eicosanol	• Hexadecanoic acids, methyl esters • 10,13-Octadecadienoic acids, methyl esters • cis-13-Octadecenoic acids, methyl esters • Methyl stearates • Oleic Acids • 1-Eicosanol • Oxacyclotridecan-2-one • 9-Tetradecenal, (Z)- • Oxalic acid, allyloctadecyl ester

Keys: PAA = Sample A Isolate A; PAB = Sample A Isolate B; PAC = Sample A Isolate C; PBA = Sample B Isolate A; PBB = Sample B Isolate B; PBC = Sample B Isolate C; PCA = Sample C Isolate A; PCB = Sample C Isolate B; PCC = Sample C Isolate C. Isolate A= *Bacillus subtilis;* Isolate B = *Lactobacillus fermentum;* Isolate C = Combination of *Bacillus subtilis* and *Lactobacillus fermentum.* Sample A = boiled for 2 h and steeped for 10 h; sample B = boiled for 4 hours and soaked for 6 hours. Sample C = boiled for 6 hours and steeped for 6 hours.

Conclusion

This study has provided valuable insights into the fermentation process of African oil beans (*Ugba*) with *B. subtilis* and *L. fermentum*, highlighting the significant impact of microbial interactions on flavour development. The findings demonstrate that co-culture fermentation not only enhance the diversities of volatile compounds but also contributes to a more complex flavour profile compared to single-culture fermentations.

The investigation into the effects of boiling and soaking times revealed their critical roles in shaping the fermentation environment, influencing microbial viability, nutrient availability, and the overall qualities of the final product. By optimizing those parameters, it is possible to standardize the fermentation process, ensuring consistent flavour and quality in *Ugba* production.

Overall, pure culture fermentations of the African oil beans produced more volatile compounds after 72 hours. Furthermore, the identification of key volatile compounds associated with both single and co-culture fermentations underscore the potential for targeted flavour enhancement in *Ugba*. This

knowledge can be leveraged to improve product acceptance and expand the culinary applications of *Ugba* in various dishes.

This study not only contributes to the understanding of the fermentations dynamics of African oil bean seeds but also lays the groundwork for future researches which would be aimed at optimizing fermentation processes and exploring the probiotic potential of the fermenting microorganisms. The findings emphasize the importance of microbial diversities and fermentation conditions in achieving high-quality fermented products, which can benefit both producers and consumers in the food industry.

Declarations

Author Contribution

Authors ECN and OJN and NIN planned, conceptualized, and constructed the protocols as well as the initial draft of the research. The laboratory tests were carried out by authors; BW and UJN, statistical analysis were conducted by authors; OAF and OCE and reviews were performed by authors OAF, ILN, ECN and OCE. Every author has read and approved the final draft. CONFLIT OF INTEREST STATEMENTThe following authors have affirmed that there are no conflict of interest in the article. Chioma N. Eluchie, Jude N. Ogbulie, Wesley Braide, Ikenna N. Nwachukwu, Arinze Francis Ofoedum, Chigozie E. Ofoedu, Judith N. Ugwoezuonu, Serah O. Alagbaoso, and Lilian N. Iroagbal on behalf of the authors affirm that-1.The manuscript has been prepared in accordance with the latest “Instruction for authors” and no conflict of interest exists.2.The article is original and has not been published previously, is not under consideration for publication elsewhere, and if accepted, it will not be published elsewhere in the same form, in English or any other language. The submission of the article has the approval of the all the authors and the authorities of the host institute where work had been carried out.3.All the authors have made substantive and intellectual contributions to the article and assume full responsibility for all opinions, conclusion and statements expressed in the articles.4.I (we) agree to abide by the comments of referees/editorial board and will modify the article as per their recommendations for publication.Name & Signature of the Researcher:Ofoedum, Arinze Francis O.A.FAddress: Department of Food Science and Technology, Federal University of Technology Owerri.Date: 13th June, 2026.

Data availability:

The raw data and chromatogram of this study can be found here.

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Figures

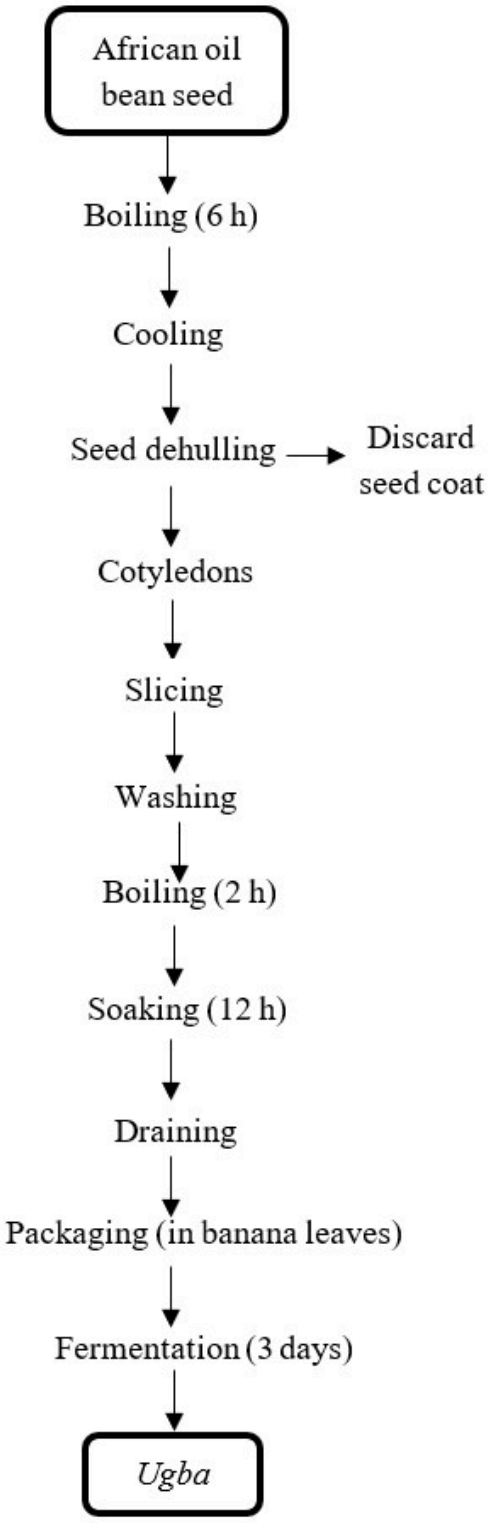

Figure 1

Production of *Ugba* (9)

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