

Effect of Isolated Cultures Fermentation on the Microbial Loads of Fermented African Oil Bean Seed (*Pentachletra macrophylla*) Products

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Abstract

The study explores the effect of isolated culture from traditionally fermented African oil bean seed on the microbial loads of pure and mixed cultures fermented "Ukana" (Ugba, African oil bean seed dessert). The isolates were morphologically and biochemically characterized. Their results as indicated in this study compared favorably to standard. Their characteristics resemble that of *Bacillus subtilis* and *Lactobacillus fermentum*. These identified isolates were used as pure and mixed cultures to ferment African oil bean seed at 24 h, 48 h, 72 h and 96 h. The result obtained indicated that total coliform and *Staphylococcus aureus* count increases across the fermentation period when AOBS was traditionally fermented, but kept decreasing as fermentation period progresses in pure and mixed cultures fermented samples. The range of the total fungal count (TFC) varied from 5.1×10^4 CfU/ml to 1.0×10^3 CfU/ml. Moreso, the number of Lactic acid bacteria recorded in this study outweighed the fungi count. The result also showed that the colony count of Lactic acid bacteria at 96 h fermentation period supersedes that of coliform, staphylococcus and fungi whereas the total coliform was higher in traditionally fermented AOBS. This result according to literature could be attributed to the production of bacitracin and organic acids, especially bacteriocins which can inhibit the proliferation of pathogenic organisms. *Bacillus subtilis* and *Lactobacillus fermentum* isolated in this research are desirable probiotic with enormous health functions.

Keywords: Isolated Culture, Microbial Load, Fermentation, Bacteriocin, Probiotic.

Introduction

The term fermentation refers to the art of biological conversion of high molecular weight substrates into simple compounds by various microorganisms such as bacteria and fungi. (Subramaniyam and Vimala, 2012). Fermented foods are prepared from plant and animal materials by processes in which microorganisms play important role in modifying the substrate physically, naturally and sensorily. Food fermentation has over the years become a part of the cultural and traditional norm among the indigenous communities in Africa. Different parts of West Africa are renowned with their own favorite food that has evolved over centuries, depending on the customs, tradition and religion (Chelule *et al.*, 2010). Many of the food fermentations are natural and/or controlled fermentation consisting of different

species and genera of yeast, fungi and/or bacteria (Fraizier and Westhoff, 2008). These microorganisms can cause desirable changes in various foods, which distinguish them from the ones that are responsible for undesirable changes, including bad flavor and spoilage (Fraizier and Westhoff, 2008).

The major pathogens, at an unprocessed form of African oil bean seed, include: *Aspergillus niger*, *Rhizopus stolonifer*, *Cladosporium herbarum*, *Penicillium spp.*, *Botryodiplodia theobromae*, and *Fusarium spp.* (Okwulehie, 2004). At the fermented stage of *Pentaclethra macrophylla* seeds (ugba), the presence of some microorganisms has been reported by Eze *et al.* (2014) and Ogbulie *et al.* (2014). This includes: *Escherichia coli* and species of *Staphylococcus*, *Klebsiella*, and *Proteus*. Also, reports exist on the possible presence of various other microorganism in ugba including *Enterococcus casseliflavus*, *E. faecalis*, *E. faecium*, *Enterobacter aerogenosa*, *Klebsilla pneumoniae*, *Salmonella enterica*, *Proteus mirabilis*, *Aeromonas sp.*, *Comamonas testosteronii* and *Clostridium sartagofum* (Okorie *et al.*, 2017). Many of these bacterial organisms, however, are part of the gastrointestinal microbiota of the human population, and hence not suggestively pathogenic in real sense. For instance, *Lactobacillus* species are habitually considered useful participants in human microbial (commensalism) ecology (Anyanwu *et al.*, 2016). However, *Escherichia coli* and species of *Staphylococcus* when ingested could be pathogenic to human. Hence the need to design a production method that will suppress the presence and proliferation of these microbes which possess adverse health impacts.

Study's Objective

The study is aimed at producing a hygienically safe African oil bean dessert (Ukana, Ugba) with high content of probiotics. The study also aimed at determining the effect of isolated cultures on pathogenic microorganisms present in traditionally fermented African oil bean dessert.

Statement of Problem

Consuming nutritionally rich but safe food can contribute to the well being of the society. "Ukana" or "Ugba" is a nutritious alkaline fermented food which is consumed without heat treatment. Several studies have reported loads of microorganisms in fermented African oil bean seed. Consuming a nutritious food with load of pathogenic microorganism can be detrimental to the health of the consumer.

The need to fill this gap, between nutritious and microbiologically safe food, prompted our study on the effect of using isolated culture for the fermentation of "Ukana".

Materials and Methods

The raw material used for this study, (African oil bean seeds), were purchased from Fiong Arang market in Akwa Ibom State, Nigeria. Pure cultures of *Bacillus subtilis* and *Lactobacillus fermentum* were developed from traditionally fermented African Oil Bean.

Microbiological Analysis of Fermented African Oil Bean Seed

African oil bean sample was prepared and transported to Microbiology Laboratory for analysis.

10g of the fermented sliced African oil bean was accurately measured and blended using mortar before mixing with 90ml of sterile water in a conical flask. Flask was labeled accordingly (Prescott, 2004).

Serial Dilution of Sample:

A serial dilution of the sample was carried out according to the method of Cheesbrough (2004). Precisely, the 10 in 90ml (aliquot) dilutions were further diluted by transferring 1ml from the aliquot into a sterile 9ml of dilution blank in a test tube, dilutions were further carried out till 10^{-3} dilution factor was obtained.

Standard Microbiological techniques described by Prescott (2004) were employed for the microbiological analysis of the African oil bean sample to determine total heterotrophic count, total staphylococcus count, total coliform count and total fungal count. 1 ml from the last dilution (10^{-3}) was transferred to a sterile Petri dish and about 20 ml of the sterile molten Nutrient agar (NA), de Man Rogosa and Sharpe agar (MRS) agar, MacConkey agar (MCA), Mannitol salt agar (MSA) and Sabouraud dextrose agar (SDA). The media are for isolation and enumeration of total heterotrophic bacteria, Lactic acid bacteria, Coliform, *Staphylococcus spp* and Fungi respectively. The plates were swirled and left on the bench to set.

The bacterial plates were incubated for 24 h at 28 °C using a Gallenkamp incubator, the MRS medium was incubated using anaerobic jar with gas pack at 28 °C for 24 to 48h.

Determination, Characterization and Partial Identification of the Microbial Flora from Traditionally Fermented Ugba

Standard Microbiological techniques described by Prescott (2004) were employed for the microbiological analysis of the fermented Ugba samples to isolate the specific organisms. Precisely, 1ml from the 4^{th} dilution was introduced into sterile petri dishes in duplicates and molten Nutrient agar, De Man Rogosa and Sharpe agar (MRS) agars were aseptically poured into the seeded plates and mixed with the inoculum. Plates were left on the bench to set (pour plate method). Nutrient agar plates were incubated at room temperature of 28 °C for 24 h and MRS agar plates at 28 °C for 48 h using anaerobic jar.

Morphology Characterization of the Isolates

The morphological characterization of the microbial cultures used for this study was done in the following manner: The colony appearance and colour were physically observed while the cell arrangement and the colony shape were observed by viewing a glass slide with a sample of smeared and stained microorganisms with the help of a microscope (Fawole and Oso, 2004).

Biochemical Characterization of the Isolates

This was done based on gram staining test, catalase test, spore test, gas production test, acid production test, alcohol production, carbohydrate utilization test using sugars like glucose, Sucrose, lactose, maltose, fructose and raffinose. The two microbial cultures were also subjected to growth in MRS agar at 15 °C, 45 °C as well as growth in nutrient Agar at room temp (30±2 °C).

Gram Staining of the Isolates

This was carried out using the method of Fawole and Oso (2004) to determine the Gram status of each of the isolates. A thin smear of each of the pure 24 h old culture was prepared on clean grease-free slides, fixed by passing over gentle flame. Each heat-fixed smear was stained with 2 drops of crystal violet solution for 60 S and rinsed with water. The smears were again flooded with Lugol's iodine for 30 S and rinsed with water, decolourized with alcohol for 15 secs and rinsed again with distilled water. They were then counter stained with 2 drops of Safranin for 60 S and finally rinsed with water and air dried. The smears were mounted on a microscope and observed under oil immersion objective lens. Gram negative cells appeared pink or red while gram positive organisms appeared purple.

Catalase test

A looful of 24 h old culture was transferred into a drop of 3 % Hydrogen peroxide solution on a clean slide with the aid of sterile inoculating loop. Gas seen as white froth indicates the presence of catalase enzyme (Cheesbrough, 2006).

Spore test

Spore tests were carried out to identify spore forming organisms. This was determined by the method of Cheesbrough (2006). A fixed smear of the isolate was made on a clean slide. It was then flooded with 5% aqueous Malachite green and stained intermittently for 1 min. The stain was washed with tap water and counter stained with 2 drops of 0.5 % aqueous safranin for 15 S. The slides were allowed to air dry, and a drop of oil immersion was put on the slide and observed under a microscope with x100 magnification. Green stain indicates presence of endospores while pink stain shows presence of vegetative cells.

Motility test

This was determined by the method of Olutiola *et al.* (2000). A 24 h old culture was picked with a sterile wire loop and streaked onto nutrient agar in petridshes. The petridishes were incubated at 37 °C for 24-48 h. Non-motile bacteria had their growth confined to the stab line with definite margins without spreading to surroundings area while motile bacteria gave diffused growth extending from the surface.

Acid production test

This was determined by the method of Olutiola *et al.* (2000). A loopful of the organism was inoculated in a test tube containing 10 ml of sterile peptone water; a dunham tube was inverted into it and incubated at 37 °C for 24 h. A change in colour was observed by dipping litmus paper into the culture solution. Presence of red colour showed acid production while absence of colour showed no acid production.

Sugar Fermentation Test

The method of Fawole and Oso (2004) was adopted. Sugar fermentation test was carried out to determine the ability of organisms to ferment sugars with production of acid and gas. Sugar indicator broth was prepared using peptone water medium containing 1 % fermentable sugar and 0.01 % phenol red. Ten milliliters (10 ml) of sugar broth was dispensed into each of the test tubes and durham tube was inverted carefully. The test tubes were autoclaved and cooled. A loopful of 24 h old culture of the test organisms each was inoculated into the different test tubes and incubated for 5 days at 36±1 °C and observed daily for acid and gas production. Yellow colouration indicated acid production while displacement of the medium in the durham tube indicated gas production.

Evaluation of the isolates ability to grow in MRS agar at 15°C.

About 15 ml of sterile MRS agar was aseptically poured inside sterile petri-dish and allowed to solidify. A loopful of the test organisms was inoculated into the dish and incubated at 15 °C for a period of 48 h under microaerophilic condition. Presence of growth showed positive result while absence of growth showed negative result.

Evaluation of the isolates ability to grow in MRS agar at 45°C.

About 15 ml of sterile MRS agar was aseptically poured inside sterile petri-dish and allowed to solidify. A loopful of the test organism was inoculated into the dish and incubated at 45 °C for a period of 48 h microaerophilic condition. Presence of growth showed positive result while absence of growth showed negative result.

Oxidase test

Tested bacterial colony was smeared on the filter paper previously saturated with freshly prepared oxidase reagent. Positive oxidase test was recorded as the development of a blue-purple colour within 10 S (Cheesbrough, 2006).

Urease test

Slanted two millilitres of urea medium which placed in bijou bottles applied for the incubated bacterial colony at room temperature. Red-pink colour in the medium was considered as a positive test for urease induction (Cheesbrough, 2006).

Methyl Red (MR) test

After adding methyl red indicator solution (TSBA, Himedia) to inoculated culturing media and incubation at 35 °C for up to 4 days, changing color to red indicate MR test positive appearance of tested bacteria (Allen *et al.*, 2016).

Purification and Maintenance of Microbial Isolates

Discrete Colonies from Primary Culture Plates were picked for Characterization. Bacterial colonies were repeatedly sub-cultured into freshly prepared Nutrient agar and MRS agar plates by streaking method and incubated for growth at optimum temperature and condition before transferring them into agar slants (Cheesbrough, 2004). The pure Isolates of bacteria were maintained on agar slant as stock and preserved in the refrigerator for further use.

Characterization and Identification of the Microbial Isolates

Bacterial isolates were characterized and identified presumptively based on their morphological, cultural and physiological characteristics. Confirmatory identification was based on biochemical reactions and molecular characterization. The following biochemical tests were carried out: Gram staining, motility, catalase, spore staining, urease, and citrate, starch hydrolysis, methyl red-voges proskaur (MR-VP) test and sugar fermentation (lactose, glucose, mannitol, maltose, galactose, xylose, fructose, and sucrose). The results obtained from the test for these isolates were collated and the identification was carried out by comparing the characteristics with known taxa using the scheme of Bergy's manual of Determinative Bacteriology (Holt's *et al.*, 1994).

Results and Discussion

Identification of *Bacillus subtilis* and *Lactobacillus fermentum*

The growth performance of the targeted unknown bacteria grown on nutrient agar (Oxoid, England) and deMan, Rogosa and Sharpe (MRS) Agar (Oxoid, CM0361 Basing stoke, UK) are shown in Plate I and II below. Plate I shows the colony morphology associated with *Bacillus Subtilis* when incubated on Nutrient agar. The colonies were creamy-white, large, flat and irregular-shape with rough wrinkled surface. Zhenxiang *et al.* (2018) observed similar growth performance of this strain on nutrient agar in his work. *Bacillus subtilis* was observed to be gray-white, round, opaque, flat and dry with medium-size. Lactic acid bacteria incubated on MRS agar were pinpointed in appearance with small and white to cream colored, convex colonies that are circular in shape (Plate II). The results obtained were collected and the identifications were ascertained by comparing the characteristics with known taxa using the scheme of Bergy's Manual of Determinative Bacteriology (Holt's *et al.*, 1994). Morphological screening result compared to the Bergy's Manual suggested that the identity of the organisms were *Bacillus subtilis* and *Lactobacillus fermentum*. Several studies by Sana *et al.* (2022) and Koirala and Anal (2021) also support the identity of these microorganisms.

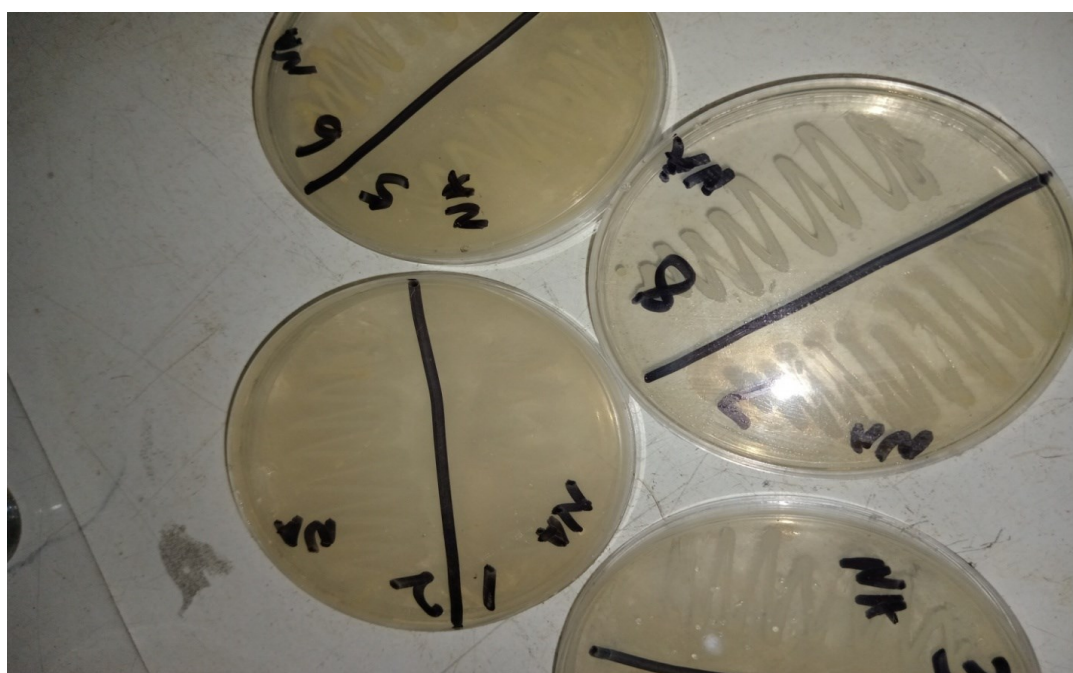


Plate I: Colony Morphology of *Bacillus subtilis*



Plate II: Colony Morphology of *Lactobacillus fermentum*

Gram-stained Bacterial Smears

The reactions of the two organisms to gram staining are presented in plates III and IV. Gram positive Bacilli capable of spore formation were observed in plate III. The Lactobacilli appeared flat surface with rod shaped gram-positive non-spore forming cells (plate IV). They were gram positive rods, small single rectangular cells with square edges, arranged as single cells. This result aligns with the standards and also confirmed the identity of *Bacillus subtilis* and *Lactobacillus fermentum*.

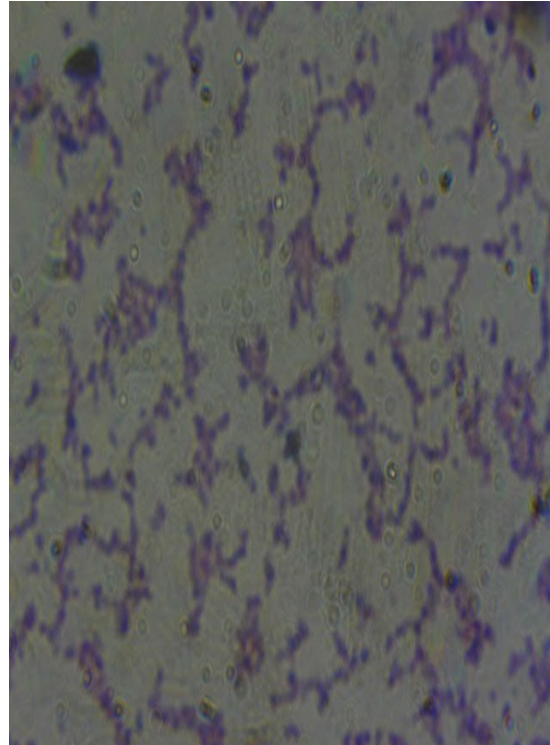
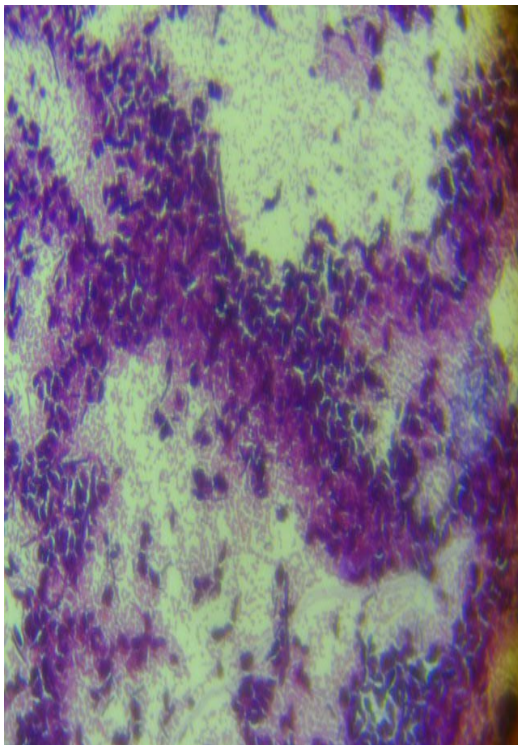


Plate III: Gram Stain View of *Bacillus subtilis*. x100

x40

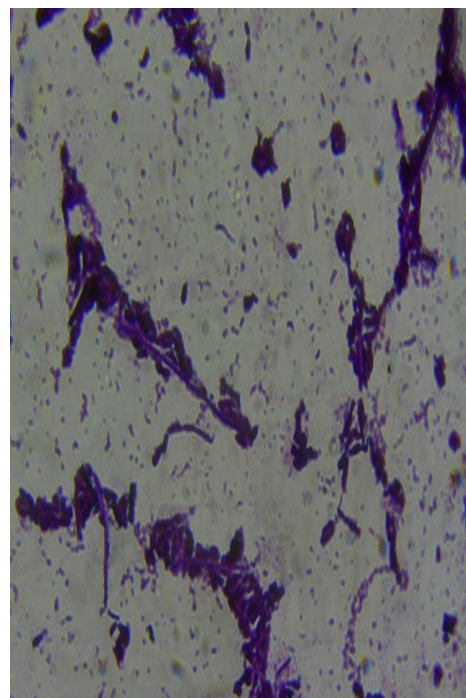
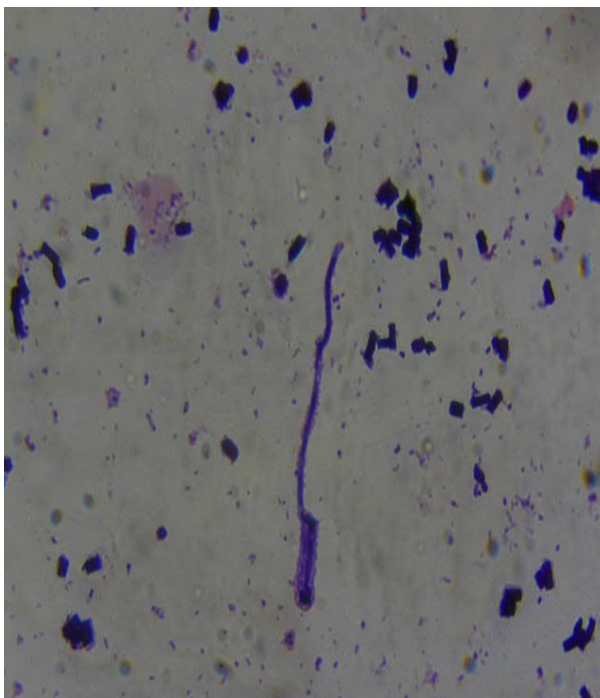


Plate IV: Gram-Stained view of *L. fermentum* x100

x40

Results of Biochemical Tests

The bacterium grown on nutrient agar fermented glucose and maltose for acid production, but not lactose. Table 1 showed that the isolate was found to be positive to catalase and

citrate test but negative to oxidase and urease tests. The VP test was positive, whereas the methyl red test was negative. The organism possessed motility property due to the presence of flagella. The bacterium was found to be catalase and nitrates positive, hence its ability to hydrolyze starch and also reduce nitrates to nitrites. This typically suggests the presence of *Bacillus subtilis*. *Lactobacillus fermentum* incubated on MRS agar produced gas from glucose. It grew well at 45°C but poorly at 15°C, in accordance with Bergey's Manual of Bacteriology (Holt's *et al.*, 1994) reports. It is also catalase, oxidase, VP and citrate negative while urease and methyl red tests were positive. However, performed morphological colony characterization and biochemical tests to identify our African oil bean seed degradation isolates were considered a traditional identification methods (Udgire *et al.*, 2015), hence the need for molecular characterization of the starter cultures for fermenting African oil bean seed sourced from Fiongan market, in the future studies.

Table 1: Biochemical characterization and identification of bacteria used for starter culture fermentation of Ugba

Gram Rxn	Shape	Catalase	Coagulate	Motility	Starch hydrolysis	Citrate test	Urease test	MR	VP	Spore formation	H ₂ S	Oxidase	Haemolysis	Nitrate Reduction	Lipase	Galactose	Glucose	Maltose	Lactose	Fructose	Sucrose	Mannitoe	Xylose	Probable org.
+ve	Rod	+	-	+	+	+	-	-	+	+	-	-	-	+	-	-	AG	A	-	A	-	-	A	<i>Bacillus subtilis</i>
+ve	Thick rod	-	-	-	-	-	+	+	-	-	-	-	-	-	-	AG	-	A	A	A	-	-	-	<i>L. fermentum</i>

Microbial Load of Fermented African Oil Bean Seed

Analyses on traditional and pure starter culture fermented African oil bean samples revealed the association of both bacterial and fungal isolates. Total count of coliform, staphylococcus, fungus, Lactic acid bacteria and heterotrophic bacteria were all carried out at various levels of fermentation and reported in tables 2 to 4 and presented in figures 1 to 4.

The result indicated that total heterotrophic bacteria count (THBC) ranges from 2.30×10^5 CfU/g in *Bacillus subtilis* fermented African oil bean seed (AOBS) at 72 h to 1.0×10^4 CfU/ml in cooked unfermented AOBS. Result also showed that total heterotrophic bacteria count (THBC) was higher compared to coliform count, Staphylococcus count, LAB count and fungal count. This is not surprising, as all the microbes isolated in this study are aerobes. This is in agreement to the study conducted by by Eluchie *et al.* (2021).

Total heterotrophic bacteria count increases across the fermentation periods in traditionally fermented African oil bean seed, with its peak at 96 h (2.21×10^5 CfU/ml) (fig 1). However, the trend was different when AOBS was fermented with pure starter cultures. For instance, the THBC of AOBS fermented with *Bacillus subtilis* increases at 24 h through 72 h and then drops at 96 h (Fig 2). Similar trend was observed when African oil bean seed was fermented with mixed culture of *Bacillus subtilis* and *Lactobacillus fermentum* (Fig 4), but there was an initial increase in THBC of AOBS fermented with *Lactobacillus fermentum* upto 48 h before a sharp decrease was observed at 72 h through 96 h. The decline in the population of total heterotrophic bacteria counts in pure starter culture fermentation with increase in fermentation time indicated the dominance of the desirable probiotics. Moreso, the proliferation of these desired microbes will lead to the increased production of bacteriocin (eg. lactic acid) which will inhibit the growth of undesired aerobes (Onwuako *et al.*, 2014).

Total Heterotrophic bacteria count defines the population of bacterial colonies produced on an agar-based medium under defined incubation temperature and time. They include all bacteria that use organic nutrients for growth. The values observed in this study are within its range in food. Allen, *et al.* (2004) stated that the upper range of THBC populations in drinking water average 5000 – 10,000 cfu/ml while its populations in food are consistently log concentrations higher (Wadhwa, *et al.*, 2002). They also stated that naturally occurring bacteria (THBC or autochthonous flora) do not have virulence factors, making their numbers in drinking water or food substance irrelevant to health risk except in the most severely immune compromised sub populations, which are fully aware of their medical condition and need to exercise appropriate dietary and other preventive measures. (Allen, *et al.*, 2004).

Total coliform count increases across the fermentation period when AOBS was traditionally fermented (Figure 1). However, the colony forming units of total coliform kept decreasing as fermentation period increases in pure and mixed culture fermented samples. The plots in Figure 2 to 4 showed that the TCC gradually reduces across the fermentation periods when AOBS were fermented with pure and combined cultures of *Bacillus subtilis* and *Lactobacillus fermentum*. This could have resulted from bacteriocin and other microbial metabolites produced from lactic acid bacteria and fatty acid. The dominance of *Bacillus* and *Lactobacillus* species could be attributed to the production of bacitracin and organic acids, especially bacteriocins (Onwuako *et al.*, 2014). The antibiotic may have inhibited the growth of other microbes and hence their disappearance as fermentation progresses (Oguoke and Aririatu, 2004). Strict hygiene should always be observed during production ugba.

Total Staphylococcus count showed a decreasing trend through all the fermentation periods (Fig. 2-4), except, when AOBS were traditionally fermented (Fig 1). The growth of Staphylococcus was visible at 24 h and 48 h fermentation periods but later disappeared when AOBS were fermented with single and mixed cultures of *Bacillus subtilis* and *Lactobacillus fermentum* (Fig 2, 3 and 4). This agrees succinctly with Onwuako *et al.* (2014).

Moreso, the bean seeds were boiled for hours before fermentation, the trace of *Staphylococcus* found could not have originated from the beans. The bacteria were probably introduced through the air, water, utensils, leaves used in wrapping or by handling during the preparatory stages (Odunfa and Oyeyiola, 1985). It has also been reported that *Staphylococcus* are more commonly associated with the skin and hence are easily disseminated through handling; thus, the organism can gain entry into the fermented AOBS by direct contact with human skin or air droplets from sneezing, hence strict hygienic practices must be adhered to during production processes. Ogueke *et al.* (2010) and Okorie and Olasupo, (2013a) reported that *Staphylococcus* is involved in the fermentation of Ugba (their number decreases as fermentation progresses). However, the result from this study when considering pure single and mixed cultures fermentation seems to be better.

Lactic acid bacteria (LAB) count was highest in *Bacillus subtilis* fermented AOBS sample (1.7×10^5 CfU/ml) at 48 h and lowest in cooked unfermented sample (4.0×10^3 CfU/ml). *Bacillus* has severally been reported as the major microorganism that is responsible for the physicochemical and organoleptic features of Ugba (Enujiugha, 2009). LAB maintain an increasing pattern across the four fermentation days in traditionally fermented and mixed pure culture fermented samples with the peak value obtained at 96th h (Fig 1 and 4). This agrees with Okorie *et al.* (2017) who opined that Lactic acid bacteria persist until the end of the fermentation, with numbers increasing throughout the period of fermentation. However, a sigmoidal trend was observed in Figures 2 and 3, when AOBS were fermented with single cultures of *Bacillus subtilis* and *Lactobacillus fermentum*. The increase in the LAB count with increase in fermentation period of the products derived from mixed culture fermentation shows the benefit of the symbiotic relationship that exist between *Bacillus subtilis* and *Lactobacillus fermentum*.

The traditionally fermented AOBS at 96 h recorded the highest count of fungi while the cooked unfermented sample had the least. The range of the total fungal count (TFC) varied from 5.1×10^4 CfU/ml to 1.0×10^3 CfU/ml. The TFC of traditionally fermented AOBS were found to build up at 24 h but slowly declined at 48 h and then slowly increases through 96 h. The trend was however different when AOBS was fermented with *Bacillus subtilis*. The plot in Figure 2 showed an increase in the TFC at 24 h through 48 h followed by a sharp decline to 96th h.

The bacteriocin secreted by *Bacillus* likely inhibited the growth of the fungi. This is the reason why the number of LAB counted outweighed the fungi count. This result agrees with Enujiugha (2009) who found *Bacillus* to persist until the end of the fermentation, with numbers increasing throughout the period of fermentation while the numbers of other microbes decreased after 24 h of fermentation (Fig 2). Figures 3 and 4 indicated the slow growth of fungi through the fermentation periods.

In summary, it has been observed from the result presented in Figures 2- 4 that the colony count of Lactic acid bacteria at 96 h fermentation period supersedes that of coliform, staphylococcus and fungi whereas the total coliform was higher in traditionally fermented AOBS. This indicated the positive impact of pure starter culture fermentation on AOBS products. The dominance of *Bacillus* and *Lactobacillus species* towards the end of the

fermentation period could be attributed to the production of bacitracin and organic acids, especially bacteriocins (Onwuako *et al.*, 2014). The antibiotic may have inhibited the growth of these other microbes and hence their disappearance towards the end of the fermentation (Oguoke and Aririatu, 2004).

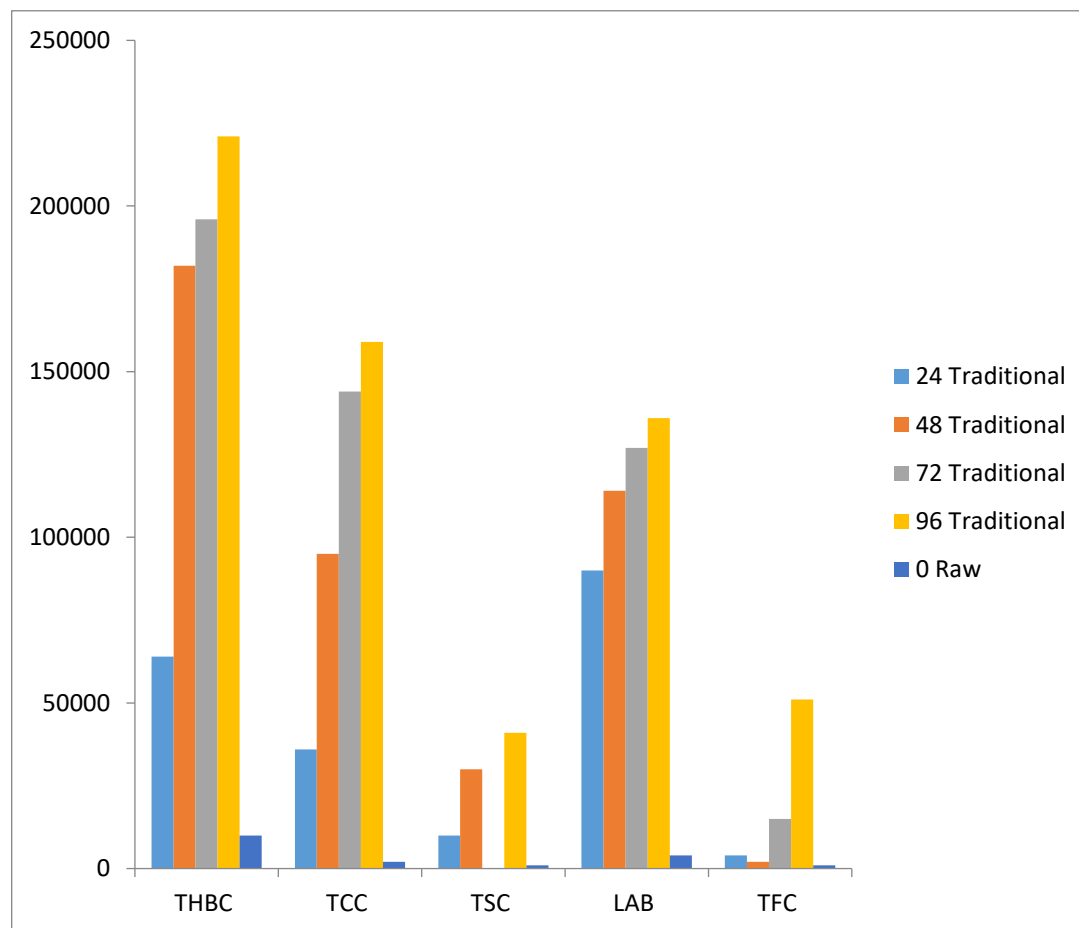


Fig.1: Microbial load of raw and traditionally fermented African oil bean seed
 THBC = Total heterotrophic bacteria count; TCC = Total coliform count; TSC =Total Staphylococcus count; LAB = Lactic acid bacteria; TFC = Total fungal count

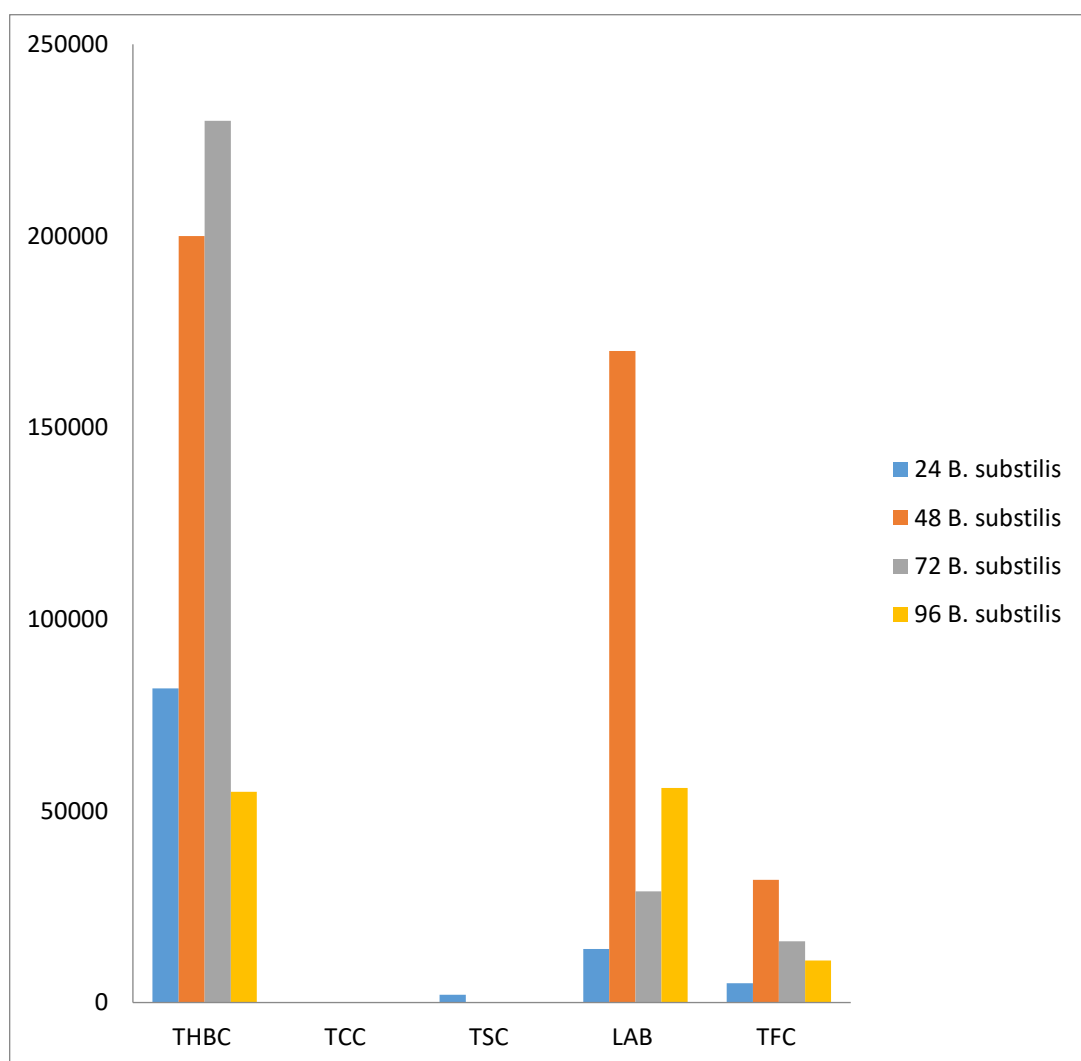


Fig.2: Microbial load of fermented African oil bean seed using *Bacillus subtilis*
 THBC = Total heterotrophic bacteria count; TCC = Total coliform count; TSC = Total Staphylococcus count; LAB = Lactic acid bacteria; TFC = Total fungal count

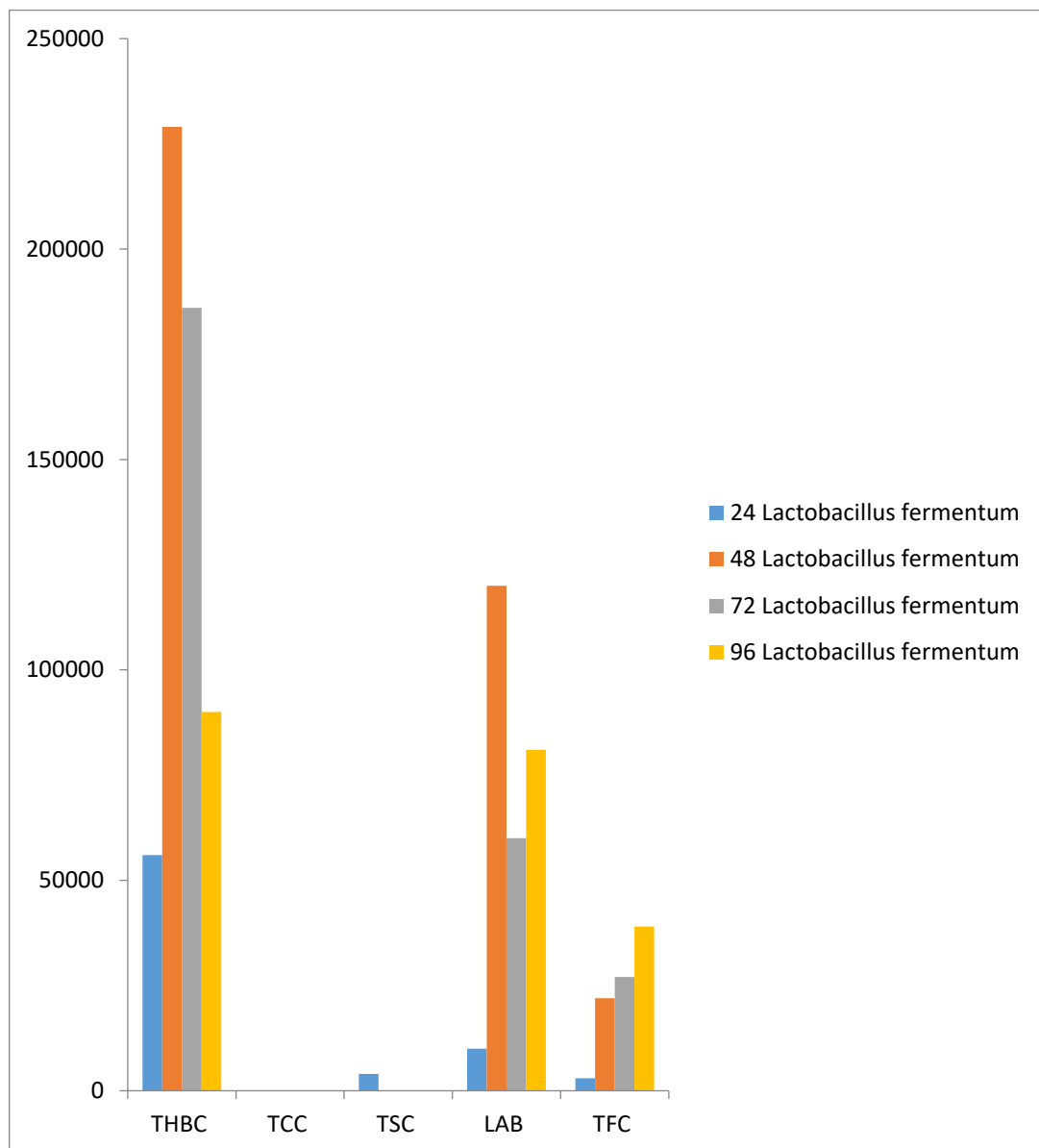


Fig. 3: Microbial load of fermented African oil bean seed using *Lactobacillus fermentum*
 THBC = Total heterotrophic bacteria count; TCC = Total coliform count; TSC = Total Staphylococcus count; LAB = Lactic acid bacteria; TFC = Total fungal count

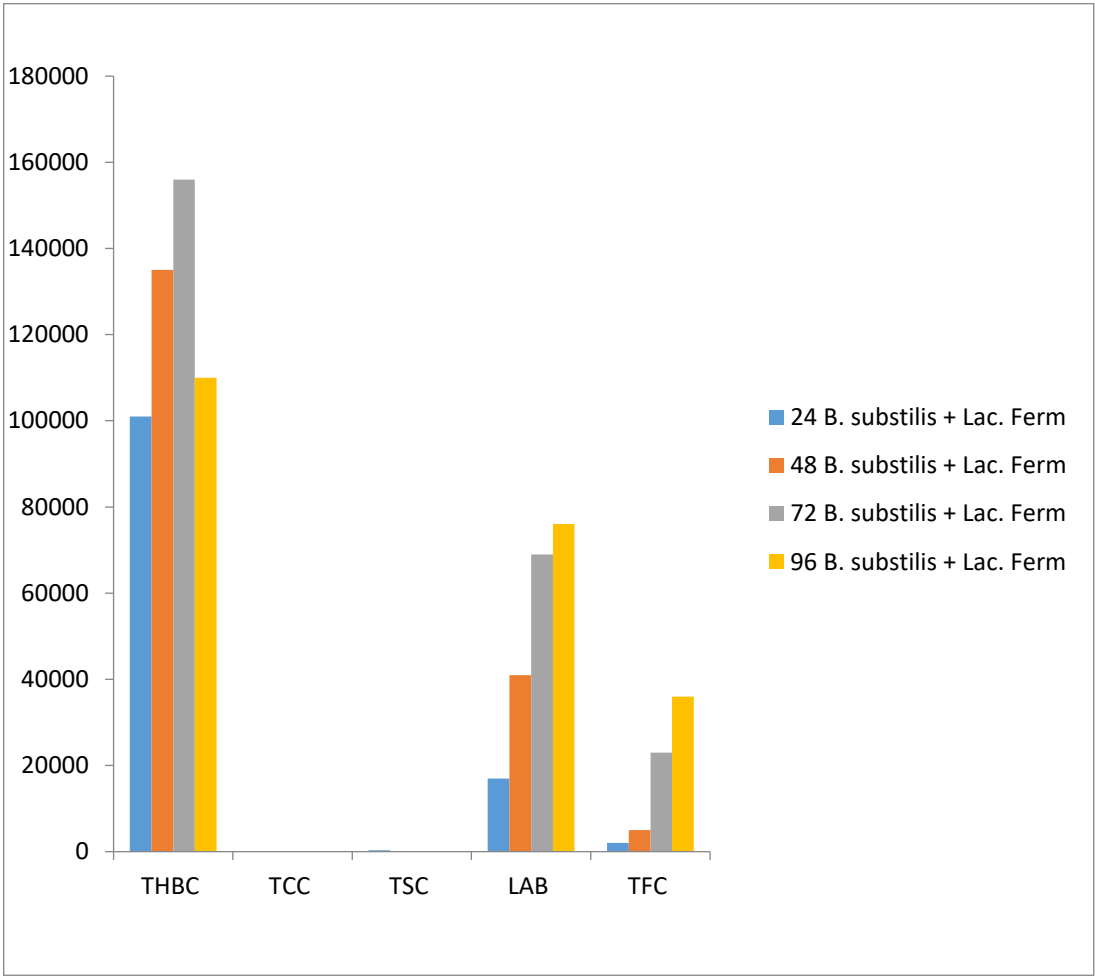


Fig.4: Microbial load of fermented African oil bean using combined isolates
THBC = Total heterotrophic bacteria count; TCC = Total coliform count; TSC = Total Staphylococcus count; LAB = Lactic acid bacteria; TFC = Total fungal count

Table 2: Performance of single isolates (CFU/g) on different growth media

TIME (h)	ISOLATES USED	THBC Nutrient Agar	TCC MacKonkeyAgar	TSC Mannitol Salt Agar	LAB De Man Rogosa Sharpe	TFC SabouraudDextrose Agar
24	<i>B.subtilis</i>	8.2×10^4	3.0×10^1	2.0×10^3	1.4×10^4	5.0×10^3
	<i>Lac. fermentum</i>	5.6×10^4	2.0×10^1	4.0×10^3	1.0×10^4	3.0×10^3
48	<i>B.subtilis</i>	2.0×10^5	2.0×10^1	0	1.7×10^5	3.2×10^4
	<i>Lac. fermentum</i>	2.29×10^5	1.0×10^1	0	1.2×10^5	2.2×10^4
72	<i>B.subtilis</i>	2.30×10^5	5	0	2.9×10^4	1.6×10^4
	<i>Lac. fermentum</i>	1.86×10^5	0	0	6.0×10^4	2.7×10^4
96	<i>B.subtilis</i>	5.5×10^4	0	0	5.6×10^4	1.1×10^4

	<i>Lac. fermentum</i>	9.0×10^4	0	0	8.1×10^4	3.9×10^4
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THBC = Total heterotrophic bacteria count; TCC = Total coliform count; TSC = Total Staphylococcus count; LAB = Lactic acid bacteria; TFC = Total fungal count

Table 3: Performance of combined isolates on different growth media

TIME (h)	ISOLATES USED		THBC Nutrient Agar	TCC MacKonkeyAgar	TSC Mannitol Salt Agar	LAB De Man Rogosa Sharpe Agar	TFC SabouraudDextrose Agar
24	B.sub + Lac.ferm		1.01×10^5	1.9×10^1	3.0×10^2	1.7×10^4	2.0×10^3
48	B.sub + Lac.ferm		1.35×10^5	2.8×10^1	8.0×10^1	4.1×10^4	5.0×10^3
72	B.sub + Lac.ferm		1.56×10^5	0	1.0×10^1	6.9×10^4	2.3×10^4
96	B.sub + Lac.ferm		1.1×10^5	0	0	7.6×10^4	3.6×10^4

THBC = Total heterotrophic bacteria count; TCC = Total coliform count; TSC = Total Staphylococcus count; LAB = Lactic acid bacteria; TFC = Total fungal count

Table 4: Traditionally fermented African oil bean

TIME (h)	THBC Nutrient Agar	TCC MacKonkeyAgar	TSC Mannitol Salt Agar	LAB De Man Rogosa Sharpe	TFC SabouraudDextrose Agar
24	6.4×10^4	3.6×10^4	1.0×10^4	9.0×10^4	4.0×10^3
48	1.82×10^5	9.5×10^4	3.0×10^4	1.14×10^5	2.0×10^3
72	1.96×10^5	1.44×10^5		1.27×10^5	1.5×10^4
96	2.21×10^5	1.59×10^5	4.1×10^4	1.36×10^5	5.1×10^4
Cooked Unfermented	1.0×10^4	2.0×10^3	1.0×10^3	4.0×10^3	1.0×10^3

Conclusions

- Fermentation improved the level of probiotic bacteria useful for improving the health conditions of consumers of the product.
- Fermenting African oil bean seed with isolated pure and mixed cultures of *Bacillus subtilis* and *Lactobacillus fermentum* under strict adherence to safety protocol will lead to the production of bacteriocins which inhibits the growth and proliferation of pathogenic microorganisms.
- Fermenting African oil bean seed with isolated cultures of *Bacillus subtilis* and *Lactobacillus fermentum* leads to reduction in the microbial load of pathogenic

microorganisms like coliforms and Staphylococcus in the product while increasing the colony of lactic acid bacteria which promotes the functional properties of the dessert.

Recommendations

- More awareness should be made on the inclusion of fermented AOBS in the diet and menu as a source of dietary probiotic bacteria.
- Production of AOBS products have been crude for ages, plans should be made to develop a system for its commercial condiment and dessert production.
- The major setback in "Ukana"s production is the inability to arrest fermentation at the desired fermentation time. It is therefore necessary to develop a system which can halt fermentation process at the prescribed fermentation period while preserving the quality of the product.

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