International Journal of Food Science and Nutrition
www.foodsciencejournal.com
ISSN: 2455-4898
Received: 01-08-2021, Accepted: 16-08-2021, Published: 02-09-2021
Volume 6, Issue 5, 2021, Page No. 01-06

Effect of starter and adjunct cultures on microbial and sensory properties of fermented African oil bean samples

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Abstract
Effect of starter and adjunct cultures on microbial and sensory properties of fermented African oil bean samples was studied. Preliminary analyses on fermentation time, temperature, pH, total titratable acidity (TTA) and organoleptic acceptability such as appearance, aroma, taste and texture to determine the best option for the addition of adjunct culture during starter fermentation of African Oil Bean with *B. subtilis*. The fermentation process lasted for a period of 96 h at 28 ± 3 ^{0}C. The total viable count, total fungal count, total *Bacillus* count, total *Lactobacillus* count, total *Coliform* count and *Staphylococcal* count of 96 h freshly fermented African Oil Bean samples were evaluated using specific media prepared according to the instructions of the manufacturers. Preliminary analyses showed that *L. fermentum* can serve as an adjunct culture to *B. subtilis* at pH of 6.30 and temperature of 36.5 ^{0}C after 24 h fermentation of African oil bean with *B. subtilis.* Microbial load of African oil bean samples increased as fermentation period progressed. Based on the microbial parameters analysed, the microbial load of sample A ranged from 0.9 x10^5 to 1.20 x 10^7CFU/g, sample B ranged from 1.0 x10^6 to 9.0 x 10^6 CFU/g, sample C ranged from1.5 x 10^6 to 1.30 x 10^7 CFU/g while sample D ranged from1.2 x 10^6 to 1.68 x 10^7 CFU/g respectively. No presence of Coliform and Staphylococcus observed throughout the period of fermentation. Sensory evaluation results showed that traditionally fermented sample was preferred most followed by African Oil Bean fermented with *Bacillus subtilis*.

Keywords: starter and adjunct cultures, microbial and sensory properties, fermentation and African oil bean

Introduction
African Oil Bean belongs to the legumi nosae family, tropical tree crop which is found in the southern rain forest zone of West Africa (Keay, 1989) [1]. African oil bean is dorminant in Nigeria as well Asia Central Africa from Senegal to South-easthern Sudan and to Angola (Keay, 1989) [1]. African oil bean is an essential food item for various traditional ceremonies where it is mixed with slices of boiled stock fish *(ugba and okporoko),* garnished with boiled vegetables and consumed by all socio-economic class. It is rich in amino acid.It is a very good food source because of its high calcium/potassium (Ca/k) ratio and could be used as alternative source of mineral supplement especially in under developed countries such as Nigeria.
Fermented foods constitute the major diet for the underdeveloped countries especially Asian country. They modify food nutritionally, improve digestion and safety as well as make it more accessible (Holzapfel, 2002) [2].
Fermentation starter cultures are preparations to aid in fermentation process in a more controlled manner in the production of various foods (Norman *et al*., 1999) [3]. Adjunct cultures are bacterial culture used in cheese, yoghurt making and other food products with the starter to produce a specific benefit including improvement in texture, flavor and nutrient content (Oxford Dictionary. 2009) [4].
Due to the prevailing population pressure in Nigeria as in other less-developed countries, there is an increase in the demand for wild exploited nutritious plant products with organoleptic appeal in the daily diet (Enujiugha and Akanbi, 2005) [5]. The common edible portions of most under-utilized plants are the seed which in some cases are cooked, roasted, eaten directly or fermented for other purposes such as Ogiri from castor bean (*Ricinus cummunis*), African locust bean (*Parkia bioglobosa)* and Ugba from African Oil Bean, etc. Consumption of foods from fermentation provide us with a lot of health benefits including improvement in nutrients, optimizing our immune system and provision of natural variety of microflora. African oil bean has bean fermented with an alkaline fermenter (*Bacillus subtilis* as starter culture*)* but not in combination with an acid fermenter (*Lactobacillus fermentum* as adjunct culture). This work aimed at evaluating the effect of starter and adjunct cultures on microbial and sensory properties of fermented African oil bean samples.

Materials and Methods
Raw materials
The raw material used for this study, African oil bean seeds were purchased from Isiala Mbano market in Imo State, Nigeria. Pure cultures of *B. subtilis* from African Oil Bean was obtained from International Institute for tropical Agriculture Ibadan and *L. fermentum* from soughdough was obtained from Pathology Laboratory, National Root Crop Research Institute, Umudike were used for this study. The chemicals obtained from Onitsha main market and Ariaria International market used for this study were of analytical grade.

Methods
Determination of the purity of starter and adjunct starter cultures.
Two microbial cultures (*Bacillus subtilis and Lactobacillus fermentum)* used for the fermentation of AOBS were evaluated for purity by subjecting them to morphological

and biochemical characterization. The two microbial cultures were also subjected to growth in MRS agar at 15^0C, 45^0C as well as growth in potato dextrose agar (PDA) at room temperature (30 ± 2^0C). Observed results were compared and validated with information from Bergey's Manual of Determinative Bacteriology. The best option was chosen based on the results of the organoleptic properties of African Oil Bean samples fermented with *B. subtilis* as starter and *L. fermentum* as adjunct.

Determination of the optimal temperature for the addition of *L. fermentum* as adjunct starter

The ideal temperature, for the addition of *L. fermentum* (microbial culture) as an adjunct to *Bacillus subtilis*; was determined by taking the temperature reading (in degree Celsius) using mercury thermometer at different periods respectively (2, 8, 12, 16 and 24 hours). The best temperature option was chosen based on the results indicated in table1 (see results section).

Preparation of traditionally fermented African oil bean.

Two and half kilograms (2.5 kg) of African oil bean seeds were sorted manually and washed to remove dust and dirt and further processed by the modification of the method of Obeta (1983) [6]. The seeds were boiled for 4 h and the hard coats were removed manually. The cotyledons were longitudinally sliced, washed and boiled again for 2 h followed by draining A quantity of 200 g of African oil bean each was put into four (4) different portions designated C_{24} h, C_{48} h, C_{72} h and C_{96} h and subjected to fermentation at room temperature for a period of 96 h. At every 24 h intervals, one sample was collected from the fermenting environment as individual sample, dried and packaged in airtight containers accordingly and kept for further analysis.

Activation of starter and adjunct cultures for the fermentation of african oil bean

A loopful of pure culture of *B. subtilis* was picked aseptically with a sterile wire loop, inoculated into 30 ml of 0.1% sterile peptone water in a test tube and incubated at temperature of 37 ^{0}C for 24 h. After incubation, 0.1 ml of *B. subtilis* culture was aseptically transferred into a sterile solidified Tryptone Soya Agar in petridish. A sterile glass rod was used to spread the culture on solidified Tryptone Soya Agar plates to ensure even distribution and subjected to further incubation at temperature of 28±2 ^{0}C for 24 h. Due to microaerophilic nature of *L. fermentum*, a loopful of it was aseptically inoculated into 25 ml of MRS broth and incubated at temperature of 30 ± 1 ^{0}C for 48 h in anaerobic jar. Thereafter, 0.1 ml each of *L. fermentum* culture suspension was aseptically transferred into different previously solidified MRS agar plates. A sterile glass rod was used to spread the culture for even distribution and incubated for a period of 48 h in anaerobic jar. Distinct colonies from different plates were used for preparation of fermentation culture.

Preparation of McFarland standard and fermentation culture media

One percent (1 %) solution of anhydrous Barium chloride ($BaCl_2$) was prepared in a beaker. One percent (1 %) solution of Sulphuric acid (H_2SO_4) was prepared in another beaker. A quantity of 0.3 ml of Barium chloride was mixed with 9.7 ml of Sulphuric acid and the absorbance measured with a spectrophotometer at 600 nm. The result was confirmed by comparing the absorbance result with that of McFarland standard chart. A loopful of 16 h old culture of a single bacterium (*B. subtilis*) from each plate was transferred into three different sterile transparent containers with 30 ml each of normal saline. The same thing was done with *L fermentum*. The addition of more organisms and dilution with normal saline stopped when the absorbance of the suspension compared favourably with that of McFarland standard. This was determined with a spectrophotometer at wavelength of 600 nm. The concentration (CFU/ml) of the starter used was determined from the McFarland standard chart by comparing the absorbance value equivalent in millimeter of the organism solution used for inoculation process.

Preliminary Investigation on the use of f *L. Fermentum* as an Adjunct to *B. Subtilis* for the Fermentation of African Oil Bean Samples.

The best option for the addition of adjunct culture was determined by aseptically adding 3 ml of each suspension of *L. fermentum* separately to 200 g quantities of African oil bean sample initially fermented with 3 ml of each solution of *B. subtilis at* 2, 8, 12, 16 and 24 h respectively. The temperature ^{0}C, pH and total titratable acidity (TTA) (designated pH1 and TTA1 were monitored for 2 – 24 h after which fermentation process continued for up to 96 h. The pH2, total titratable acidity (TTA2) and some organoleptic parameters such as appearance, taste and texture were monitored after 96 h of fermentation. Based on the fact that fermentation of African Oil Bean with *B. subtilis* and *L, fermentum* (adjunct culture) was successful which was observed based on the sensory parameters assessed, sample C (African Oil Bean sample fermented with *B. subtilis* and *L. fermentum*) was subjected to initial fermentation process for 24 h. After 24 h, *L. fermentum* inoculum at temperature of 36.5 ^{0}C and pH of 6.3 was inoculated and fermentation proceeded for a period of 96 h. Based also on the fact that *L. fermentum* (adjunct culture) grew well at temperature of 36.5 ^{0}C and pH of 6.3, sample B's (African oil bean sample fermented with *L. fermentum*) pH was also adjusted to 6.3 using 0.1N NaOH solution and at a temperature of 36.5 ^{0}C.

Preparation of Starter Culture Fermented African Oil Bean.

Sample preparation for starter culture fermented African Oil Bean was the same as the treatment given to traditionally fermented samples before the inoculation process. Based on the result obtained from preliminary analyses, three milliliters 3 ml (9.0×10^8 CFU/ml) each of *B. subtilis* inoculum was inoculated into 200 g each of African oil bean samples designated A24 h, A48 h, A72 h and A96 h (sample A at different fermentation period), 3 ml (9.0×10^8 CFU/ml) each of *L. fermentum* inoculum was aseptically inoculated into 200 g each of African oil bean samples designated B24 h, B48 h, B72 h and B96 h at pH of 6.3 and temperature of 36.5 ^{0}C and fermentation process proceeded until a period of 96 h (sample B at different fermentation period) respectively. The subsequent fermentation was done by aseptically inoculating 3 ml (9.0×10^8 CFU/ml) each of *B. subtilis* inoculum into 200 g each of the samples designated C24h through C96 h for the initial fermentation period of 24h followed by addition of 3 ml (9.0×10^8 CFU/ml) each

of adjunct culture (*L.fermentum*) inoculum also into other samples designated C48 h,C72 h and C96 h at pH of 6.3 and temperature of 36.5^{0}C and fermentation process proceeded until a period of 96 h (sample C at different fermentation period) while sample designated E0h (Cooked unfermented African oil bean sample) was subjected to drying immediately after 2 h cooking and draining for 12 min to prevent fermentation from taking place. For the fermented samples, one sample plate was collected from the fermenting environment at every 24h interval until the 96 h sample was taken as individual samples, dried, packaged accordingly and kept in airtight containers for further analysis.

Evaluation of the Microbial Load of African Oil Bean Samples Fermented at Different Periods.

All the media used for this research were prepared according to the manufacturer's instruction. A quantity of 11.75 g of plate count agar (Titan Biotech. Ltd India) used for cultivation of viable bacteria was weighed with a weighing balance into a previously labeled sterile conical flask (500 ml) followed by the addition of distilled water. The mixture was properly mixed and the flask made up to 500 ml with distilled water. The flask was covered with cotton wool and foil to prevent the content of the flask from pouring away and also gaining access to more water. Nine point seven five grams (9.75 g) of potato dextrose agar (Hair Momeid Ltd) used for cultivation of fungi, was weighed into sterile conical flask (250 ml) labeled TFC and was mixed well with 100 ml of water after which the flask was made up to 250 ml with distilled water and covered with foil and cotton wool. For cultivation of Bacillus spp, 3 % of Magnesium sulphate heptahydrate was added to 250 ml of nutrient agar prepared by weighing 7 g of nutrient agar (Fluka) into previously labeled sterile conical flask (500 ml) containing 250 ml of distilled water followed by the addition of 3 % of Magnesium sulphate heptahydrate. De Man Rogosa Sharpe agar (MRS agar) (Titan Biotech.Ltd) used for cultivation of *Lactobacillus spp* was prepared by weighing 16.775 g of MRS agar into a previously labeled sterile conical flask (500 ml). The MRS agar was dissolved with water until the flask was made up to 250 ml with distilled water and covered with cotton wool and foil. Eiosin Methylene blue agar (EMB agar) used for cultivation of Coliform bacteria was prepared by weighing 9 g of EMB agar into a previously labeled sterile conical flask and mixed well with 250 ml of distilled water and covered with cotton wool and foil. while mannitol salt agar used for cultivation of *Staphylococcus* spp was prepared by weighing 27.75g of mannitol salt agar into a previously labeled sterile conical flask and mixed well with 250 ml of distilled water and covered with cotton wool and foil. All the media were then sterilized by autoclaving at 121 ^{0}C for 15 min. Each of the sterilized media was cooled and poured separatetly into different sterile petri dishes previously labeled and allowed to solidify. One gram (1 g) of each sample was weighed into five different tubes each containing 9 ml of peptone water and labeled 10^{-1} each. The serial dilution process continued up to 10^{-5} dilution factor for each sample. The total viable count was determined by the pour plate method described by Ezeama, (2005) [7]. This was achieved by inoculating 0.1 ml each of the sample solution into different petri dishes in duplicates after which 15 ml each of sterile plate count agar was poured into different petridishes,solidified and incubated at 37 ^{0}C for 24 h, total fungal count was determined by inoculating 0.1 ml each of the sample solution into different petri dishes in duplicates after which 15 ml each of sterile potato dextrose agar was poured into different petri dishes, solidified, inverted and incubated at 25 ^{0}C for 48. Fifteen milliliters (15 ml) of medium containing a mixture of nutrient agar and Magnesium sulphate heptahydrate used for isolation of *Bacillus* spp. was poured into each of petridishes containing 0.1 ml of organism solution, solidified, inverted and incubated in an incubator (surgical sm 905 Medical England) at 37 ^{0}C for 24 h. Fifteen milliliters (15 ml) of De Man Rogosa Sharpe was poured into each of agar plates containing 0.1 ml of organism solution, solidified, inverted and incubated anaerobically under anaerobic jar for 48 h. This was used for the identification of Total *Lactobacillus* count. Eiosin methylene agar plate used for the isolation of Coliform organisms was incubated for 48 h at 37 ^{0}C while Mannitol salt agar used for isolation of *Staphylococcus* was also incubated for 48 h at 37 ^{0}C. These incubation process was carried out at with 24 h, 48 h, 72 h and 96 h samples respectively. After incubation, the bacterial colonies were observed and counted using a colony counter (Gallenkamp).

Sensory Evaluation

Sensory evaluation of African oil bean samples were prepared using method as described by Ihekoronye and Ngoddy (1985) [8]. A total of 22 semi panelists who were familiar with the quality attributes of fermented African oil bean were selected from students of the Department of Food Science and Technology, Michael Okpara University of Agriculture, Umudike.

Statistical Analysis

The data obtained from duplicate samples of the microbial analysis were calculated as mean values and expressed as colony forming unit per gram (CFU/g while sensory analysis was subjected to analysis of variance of a completely randomized design (C.R.D) using the SPSS procedure version 22 for personal computers. The treatment mean were separated using Duncan's multiple range test at significant difference of 95% confidence level ($p<0.05$).

Results and Discussion

Results of preliminary experiments to determine the best option for the use of *L.Fermentum* as an adjunct starter to *B. Subtilis*

Organoleptic properties of african oil bean samples fermented with *b. subtilis* as starter and *l. fermentum* as adjunct.

Table 1 shows the organoleptic properties of African Oil Bean samples fermented with *B. subtilis* as starter and *L. fermentum* as adjunct. Sample A5 was chosen as the best option for the use of *L. fermentum* as adjunct culture during the initial fermentation of African Oil Bean with *B. subtilis* based on some organoleptic parameters (appearance, aroma, taste and texture) analyzed. Samples A1 through A4 were not chosen because of the nature of the African Oil Bean samples obtained after the fermentation period stipulated. This may be attributed to the conditions under which the adjunct culture was added since the acid fermenter (*L. fermentum*) and alkaline fermenter *(B. subtilis*) cannot work under the same condition.

Table 1: Organoleptic properties of African Oil Bean samples fermented with *B.subtilis* as starter and *L. fermentum* as adjunct at specific periods.

Sample code	Appearance	Aroma	Taste	Texture
A1	Dark green	Bad	Bitter	Slimy
A2	Dark green	Bad	Bitter	Slimy
A3	Dull brown	Bad	Bitter	Hard
A4	Dull brown	Fair	Bitter	Hard
A5	Light brown	Good	Good	Hard

Where A1 =African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 2 h, A2 =African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 8 h, A3 =African Oil Bean sample fermented for 12 h with *B. subtilis* followed by the inoculation of adjunct culture (*L. fermentum*), A4 =African Oil Bean sample fermented for 16 h with *B.subtilis* followed by the inoculation of adjunct culture *(L. fermentum*) and A5 =African Oil Bean sample fermented initially with *B.subtilis* for 24 h followed by the inoculation of adjunct culture (*L. fermentum*)

Results of screening for optimal microbial inoculation, PH, total titratable acidity, time and temperature

Figures 1a,b, and c show the results for the initial and final pH; initial and final total titratable acidity (TTA) (g/ml) and the fermentation times (h) and temp.^{0}C for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis*. The use of L. *fermentum* as adjunct culture to *B. subtilis* for the fermentation of African Oil Bean proved effective after 24h at a temperature of 36.5 ^{0}C and pH of 6.30. Increase in temperature was observed with increase pH and decrease in. total titratable acidity (TTA) (g/ml).

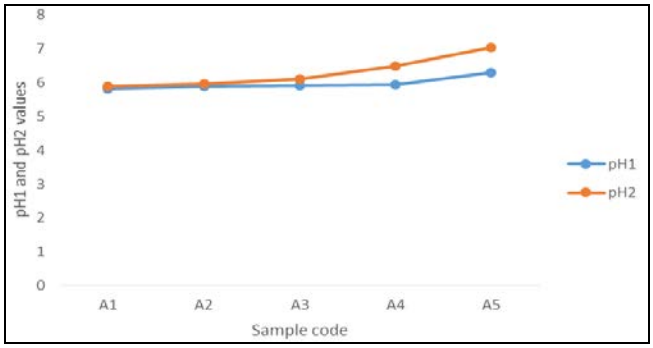

Fig 1a: Initial (pH1) and final pH plots for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

Where A1=African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 2 h, A2 =African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 8 h, A3 =African Oil Bean sample fermented for 12 h with *B. subtilis* followed by the inoculation of adjunct culture (*L. fermentum*), A4 =African Oil Bean sample fermented for 16 h with *B.subtilis* followed by the inoculation of adjunct culture *(L. fermentum*) and A5 =African Oil Bean sample fermented initially with *B.subtilis* for 24 h followed by the inoculation of adjunct culture (*L. fermentum*).

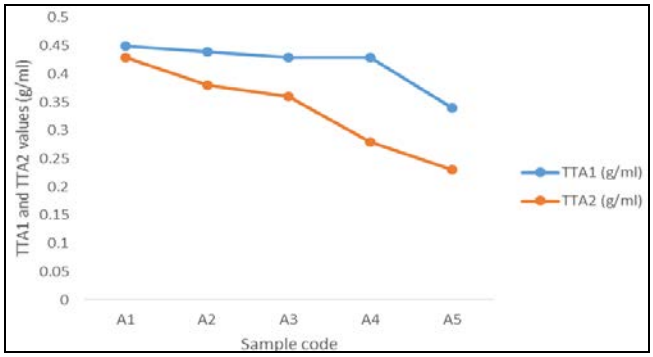

Fig 1b: Plot of the initial and final TTA for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

Where:

A1 =African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 2 h, A2=African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 8 h, A3 =African Oil Bean sample fermented for 12 h with *B. subtilis* followed by the inoculation of adjunct culture (*L. fermentum*), A4 =African Oil Bean sample fermented for 16 h with B. subtilis followed by the inoculation of adjunct culture *(L. fermentum*) and A5 =African Oil Bean sample fermented initially with *B. subtilis* for 24 h followed by the inoculation of adjunct culture (*L. fermentum*)

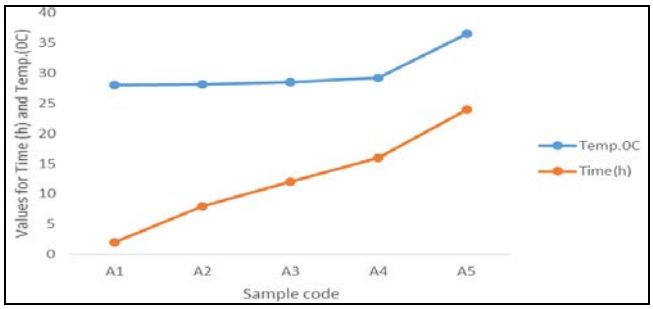

Fig 1c: Plot of values for temperature (^{0}C) and fermentation times for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

A1=African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 2 h, A2 =African Oil Bean sample fermented initially with *B.subtilis* followed by the inoculation of adjunct culture (*L. fermentum*) after 8 h, A3 =African Oil Bean sample fermented for 12 h with *B. subtilis* followed by the inoculation of adjunct culture (*L. fermentum*), A4 =African Oil Bean sample fermented for 16 h with B.subtilis followed by the inoculation of adjunct culture *(L. fermentum*) and A5 =African Oil Bean sample fermented initially with *B.subtilis* for 24 h followed by the inoculation of adjunct culture (*L. fermentum*)

Microbial Load of African Oil Bean Samples Fermented at Different Periods.

The microbial load of African oil bean samples fermented at different periods as shown in table 2 indicates that total viable count increased with increase in period of fermentation in all the samples analysed. However, their values fell within the standard or permissible level (30-300

CFU/g) stipulated by International standard organization (ISO). This is based on the fact that the African Oil Bean samples were produced under aseptic condition. Their values ranged from 4.6 x 10^6 to 1.20 x 10^7 CFU/g for sample A (African Oil Bean sample fermented with *Bacillus subtilis*), 1.0 x 10^6 to 9.0 x 10^6 CFU/g for sample B (African Oil Bean sample fermented with *Lactobacillus fermentum*), 4.8 x 10^6 to 1.30 x 10^7 CFU/g for sample C (African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum)* and 4.8 x 10^6 to 1.68 x 10^7 CFU/g for sample D (African Oil Bean sample fermented traditionally). Based on all the parameters micribiologically analysed, the microbial load of sample A ranged from 0.9 x 10^5CFU/g, sample B ranged from The presence of fungi was observed in all the samples after 24 h period of fermentation except in sample C (African Oil Bean sample fermented traditionally) where fungi were present from 24 h to 96 h period of fermentation. This may be attributed to the fact that fermentation of African Oil Bean cannot be achieved without the help of fungi especially yeast as indicated by Mba *et al*., (2010). No presence of lactic acid bacteria was observed in sample A (African Oil Bean sample fermented with *Bacillus subtilis).* No presence of *Bacillus spp* was observed in sample B (African Oil Bean sample fermented with *Lactobacillus fermentum*). This may be as a result of the nature of the starter culture involved in the fermentation of the two samples. Total Coliform count and total Staphylococcal count were insignificant in all the samples analysed. This is an indication of food safety.

The Result of Sensory Attributes of African Oil Bean Samples.

The result of sensory characteristics of African oil bean samples showed that significant difference ($p<0.05$) existed between samples A(African oil bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum)* and sample D(African oil bean sample fermented with *Lactobacillus fermentum*) based on appearance as shown in table 3. No significant difference ($p>0.05$) was observed among samples A, C and D (African Oil Bean sample fermented with *Bacillus subtilis,* African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum* and African Oil Bean sample fermented traditionally). The difference in appearance observed in sample B (African Oil Bean sample fermented with *Lactobacillus fermentum*) may have been caused by the starter involved in the fermentation process.

Table 3. Shows that sample D (African oil bean sample fermented traditionally) rated highest (7.14) in terms of taste. This may be attributed to the type and number of micro-organism involved in the fermentation process. According to Ohiri and Bassey (2017) [10], fermentation of ugba improved its taste and reduced the lipid content to a minimal level. Significant difference($p<0.05$) was observed between samples B and D (African oil bean sample fermented with *Lactobacillus fermentum* and African oil bean sample fermented traditionally*)*.This may be due to the fact that African oil bean sample undergoes alkaline fermentation. Moreso,the nature of the organisms involved in the fermentation may be a contributing factor.The difference in Taste observed in Sample B(African oil bean sample fermented with *Lactobacillus fermentum*) may be attributed to the organism that was involved in the fermentation process.

No significant difference($p>0.05$) was observed among the African oil bean samples A to D(African oil bean sample fermented with *Bacillus subtilis,* African oil bean sample fermented with *Lactobacillus fermentum*, African oil bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum* and African oil bean sample fermented traditionally) in terms of texture. Their values ranged from 6.27 to 6.95.

In terms of aroma, sample D (African Oil Bean sample fermented traditionally) rated highest (7.77). Significant difference ($p<0.05$) was observed between sample A (African Oil Bean sample fermented with *Bacillus subtilis)* and samples B and C (African Oil Bean sample fermented with *Lactobacillus fermentum* and African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum*). The aroma of ugba was attributed to the presence of methyl esters of various long chains fatty acids as reported by Nwokeleme and Ugwuanyi, (2015). No significant difference ($p>0.05$) existed between samples A and C (African Oil Bean sample fermented with *Bacillus subtilis* and African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum*). Their values ranged from 5.27 to 7.77.

Based on overall acceptability, samples A and D (African Oil Bean sample fermented with *Bacillus subtilis* and African Oil Bean sample fermented traditionally) were preferred most followed by sample B (African Oil Bean sample fermented with *Lactobacillus fermentum)* and sample C (African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum*) as shown in table 3.3. Significant difference ($p<0.05$) was observed between sample D (African Oil Bean sample fermented traditionally) and samples B and C (African Oil Bean sample fermented with *Lactobacillus fermentum* and African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum*). This may be due to the type of organisms involved in their fermentation process for *Bacillus* spp have been implicated as the dorminant organisms in African oil bean fermentation.

Table 2: Changes in the microbial load of African Oil Bean samples fermented at different periods.

Microbial count in CFU/g	Sample code	Period of fermentation (h)			
TVC	A	24 4.6 x 10^6	48 7.9 x 10^6	72 1.00 x 10^7	96 1.20 x 10^7
TFC	A	$<10^1$	0.9 x 10^5	1.3 x 10^6	1.7 x 10^6
TLC	A	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TB^{1}C	A	4.5 x 10^6	5.4 x 10^6	8.7 x 10^6	1.01 x 10^7
TCC	A	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TVC	B	1.0 x 10^6	3.8 x 10^6	5.5 x 10^6	9.0 x 10^6
TFC	B	$<10^1$	5.0 x 10^5	1.8 x 10^6	3.3 x 10^6
TLC	B	9.0 x 10^4	3.2 x 10^6	3.7 x 10^6	5.5 x 10^6

TB[1]C	B	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TCC	B	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TVC	C	4.8×10^6	8.8×10^6	1.25×10^7	1.30×10^7
TFC	C	$<10^1$	2.0×10^6	2.8×10^6	3.3×10^6
TLC	C	$<10^1$	1.5×10^6	3.8×10^6	4.5×10^6
TB[1]C	C	4.6×10^6	4.9×10^6	6.0×10^6	5.0×10^6
TCC	C	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TVC	D	4.8×10^6	8.8×10^6	1.35×10^7	1.68×10^7
TFC	D	1.2×10^6	2.0×10^6	2.4×10^6	3.2×10^6
TLC	D	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TB[1]C	D	6.6×10^6	7.0×10^6	9.0×10^6	1.10×10^7
TCC	D	$<10^1$	$<10^1$	$<10^1$	$<10^1$
TSC	D	$<10^1$	$<10^1$	$<10^1$	$<10^1$

*All values are mean counts (CFU/g)

TVC-Total viable count, TFC-Total fungal count, TLC-Total Lab. count, TB[1]C-Total *Bacillus* count, TCC-Total coliform count and TSC-Total *Staphylococcal* count.
A- African Oil Bean sample fermented with *Bacillus subtilis*
B- African Oil Bean sample fermented with *Lactobacillus fermentum.*
C- African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum.*
D- African Oil Bean sample fermented traditionally

Table 3: The result of sensory attributes of African Oil Bean samples fermented at different periods

	Parameters				
Sample code	**Appearance**	**Taste**	**Texture`**	**Aroma**	**Overall acceptability**
A	5.77^{ab}±2.09	6.23^{a}±1.63	6.73^{a}±1.35	6.09^{a}±2.09	6.91^{ab}±1.66
B	5.18^{b}±1.82	5.00^{b}±2.12	6.95^{a}±1.50	5.27^{b}±2.16	5.95^{b} ±2.06
C	6.68^{a}±1.56	6.14^{a}±1.52	6.41^{a}±1.60	6.14^{b}±1.98	6.64^{b}±1.47
D	6.82^{a}±2.22	7.14^{a}±1.49	6.27^{a}±1.67	7.77^{a}±0.97	7.68^{a}±1.29

Means not followed by the same superscript in the same column are significantly different ($p<0.05$).
A- African Oil Bean sample fermented with *Bacillus subtilis*
B- African Oil Bean sample fermented with *Lactobacillus fermentum*
C- African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum*
D- African Oil Bean sample fermented traditionally

Conclusion

The study revealed that *L. fermentum* can serve as a good adjunct culture to *B. subtilis* starter for fermentation of African oil bean. Microbial load was observed to increase with increase in period of fermentation.Sensory evaluation of the fermented African oil bean samples showed that traditionally fermented African oil bean sample was preferred most followed by African oil bean fermented with only *B. subtilis* starter.

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