International Journal of Food Science and Nutrition
ISSN: 2455-4898; Impact Factor: RJIF 5.14
Received: 25-01-2020; Accepted: 26-02-2020; Published: 29-03-2020
www.foodsciencejournal.com
Volume 5; Issue 2; 2020; Page No. 63-70

Evaluation of the effect of fermentation of African oil bean (*Pentaclethra macrophylla*) using bacillus subtilis as starter and Lactobacillus fermentum as adjunct on the vitamin and mineral contents of the seeds

Nwanagba NL[1*], Ojimelukwe PC[2], Ezeama CF[3]
[1-3] Department of Food Science and Technology, College of Health Sciences and Management Technology, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria

Abstract
Effect of starter culture fermentation and the use of an adjunct starter on the vitamins and mineral composition were investigated. Preliminary experiments were carried out to determine the best conditions (pH, temperature and organoleptic properties) for the use of *Lactobacillus fermentum* as an adjunct to *Bacillus subtilis* in fermenting African Oil Bean seeds. African Oil Bean was processed and subjected to different fermentation methods: (a) use of *B subtilis* as a monoculture (b) use of *L. fermentum* as monoculture culture (c) use of *B. subtilis* and *L. fermentum* as mixed culture (d) traditional/chance fermentation. The fermentation process lasted for a period of 96 h. After drying the 96 h fermented African Oil Bean samples at 50 ^{0}C for 3 h, the samples were evaluated for vitamin (B_1, B_2, B_3, C, A and E) and mineral (Ca,P, Fe and Zn) composition. Preliminary experiments 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.* Fermentation was observed to increase the mineral and vitamin contents of African Oil Bean samples. The calcium content ranged from 212 to 256mg/100g, magnesium content ranged from 176 to 219mg/100g, phosphorus content ranged from 186 to 234mg/100g, Iron content ranged from 9.25 to 10.58 mg while zinc content ranged from 5.33 to 9.60mg/100g for all the samples.

Keywords: African oil bean, microbial load, mineral, vitamin, starter and adjunct cultures

1. Introduction

In fermentation, microorganisms convert major nutrients to simple compounds (Subramaniyam and Vimala, 2012) [1]. Fermented foods make a major contribution to dietary staples in numerous countries across Asia, Latin America (FAO, 1998) [2]. Fermented foods are prepared from both plant and animal materials using processes in which micro-organisms play active roles in the physical, nutritional and organoleptic modifications of the starting material (Aidoo, 1994) [3]. Its techniques offer a lot of benefits in improving the food status of many people in the Universe particularly the marginalized and vulnerable groups. This is achieved through improved food preservation, increasing the range of raw materials that can be used to produce edible food products and removing antinutritional factors to make food safe to eat.

Fermented foods are rich in probiotics. Researchers have demonstrated how the bacteria in the gut form the foundation for physical, mental and emotional well-being. Consumption of fermented foods provides 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 belongs to the Leguminosae family. It is a tropical tree crop which is found in the southern rain forest zone of West Africa (Keay, 1989) [4]. African Oil Bean seed undergoes fermentation which converts it from an inedible seed to a protein rich food. The seeds in their natural form contain toxic alkaloids and saponins and are bitter to taste. The fermentation of the seeds makes the product nutritious, palatable and non-toxic (Enujiugha and Akanbi, 2005) [5]. 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) [6]. Adjunct cultures are bacterial culture used in cheese and yoghurt making with the starter to produce a specific benefit including improvement in texture, flavor and nutrient content (Oxford Dictionary. 2009). Researchers have indicated that fermentation brings about nutritionally-better product than the raw seeds (Achinewhu and Ryley, 1986; Enujiugha and Olagunde, 2001) [7, 8]. This study aims at evaluating the microbial load, mineral and vitamin composition of African oil bean samples fermented with starter (*Bacillus subtilis*) and adjunt (*Lactobacillus fermentum*).

2. Materials and Methods

2.1 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* for the fermentation of African Oil Bean was obtained from International Institute for Tropical Agriculture (IITA) Ibadan, Nigeria 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.

2.2 Methods

2.2.1. Determining 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 biocemical characterization in the following manner: The colony appearance, colony colour and the colony size 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. Biochemical characterization was also carried out 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 15^0C, 45^0C as well as growth in potato dextrose agar (PDA) at room temp (30 ± 2^0C). Observed results were compared and validated with information from Bergey's Manual of Determinative Bacteriology. The optimal temperature for the addition of *L. fermentum* (microbial culture) as an adjunct was determined by taking the temperature reading in degree celcius using mercury thermometer inserted in the bore hole of the fermenting substrate at different periods respectively (2, 8, 12, 16 and 24 hours). The best temperature 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 optimal temperature for the addition of *L. fermentum* (microbial culture) as an adjunct was determined by taking the temperature reading (in degree celcius) using mercury thermometer inserted in the bore hole of the fermenting substrate at different periods respectively (2, 8, 12, 16 and 24 hours). The best temperature 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 as shown in table1.

2.2.2. Preparation of traditionally fermented African oil bean.

Two and half kilograms (2.5 kg) of African oil bean seeds were sorted manually to remove defective seeds and washed to remove dust and dirt. The African oil bean seeds sample was further processed by the modification of the method of Obeta (1983) [9]. 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. After draining, two hundred grams (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.

2.2.3 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 intodifferent 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.

2.2.4 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.

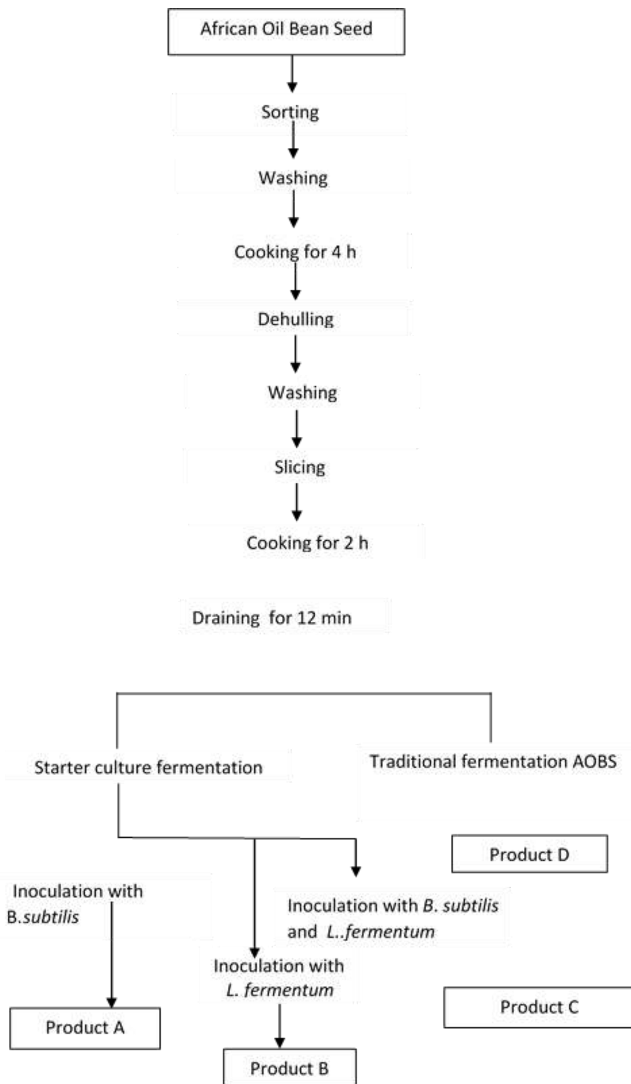

Fig 1: Flowchart for the processing of African Oil Bean samples. Fermentation was for 24, 48, 72 and 96h respectively

2.3 Preliminary investigation on the use of *L. fermentum* as an adjunct to *B. subtilis* for the fermentation of African oil bean sample

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 to96 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.

2.4 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 x 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 x 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 x10^{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 x 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.

2.5 Determination of vitamin contents of African oil bean samples

2.5.1. Determination of vitamin B_1 (Thiamine)

Vitamin B_1 of the samples was analysed by the scalar colourimetric method as described by Okwu and Josiah (2006) [10].

Five-gram (5 g) portions of the samples were extracted by homogenizing them in 50ml of normal ethanol sodium hydroxide solution. The homogenate was filtered through Whatman's No 42-filter paper and the residues were washed with the extracts treated with 10 ml of dilute Potassium ferrocyanide in a 50 ml volumetric flask. A standard vitamin B_1 solution and the extracting solution were used to set up the standard and reagent blank respectively. Both were treated as described above. The absorbance was read in a spectrophotometer (model 752) at a wavelength of 360 nm with the reagent blank set at zero. The formular given as follows was used to calculate the vitamin B_1 content of samples.

$$\text{Thiamine (mg/100g)} = \frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{Vf}{Va} \times D \quad \text{........Eqn 3.6}$$

Where:

W = weight of sample

AU = Absorbance of sample.
AS = Absorbance of standard
C = Concentration of standard (mg/ml)
VF = Volume of extract
Va = Volume of extract analysed
D = Dilution factor where applicable

2.5.2 Determination of vitamin B_2 (Riboflavin)

The Riboflavin content of the sample was determined by the method of Okwu and Josiah (2006) [10].

Five grams (5 g) of the sample was dispersed in 2.0 ml of 50% ethanol solution and shaken for 1h to extract riboflavin.This was filtered into a 100 ml flask; a 20 ml aliquot of the extract was pipetted into 50 ml volumetric flask.Twenty milliliters (20 ml) of 5% Potassium permanganate solution and 20 ml of 30% Hydrogen peroxide were added and heated on a hot water bath for 30 min. Two milliliters (2 ml) of 4% Sodium sulphate solution was added until a pale yellow colour developed. This was made up to 50 ml mark and the absorbance measured at 510 nm using a spectrophotometer. The vitamin B_2 content was calculated as follows:

$$\text{Riboflavin (mg/100g)} = \frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{Vf}{Va} \text{ ....Eqn 3.7}$$

Where,
W = weight of sample analysed
AU = Absorbance of the test sample.
AS = Absorbance of standard vitamin B2 solution
VF = Total Volume of filtrate
Va = Volume of filtrate analysed
C = Concentration of standard (mg/ml)
D = Dilution factor where applicable

2.5.3 Determination of vitamin B_3 (Niacin)

Niacin content was determined using AOAC (2006) [11] method. Two mililitres (2 ml) of each sample was treated with 50 ml in Sulphuric acid and shaken for 30 min, 3 drops of ammonia solution were added to the sample and added into a 50 ml volumetric flask and 5 ml of Potassium ferrocyanide was added. This was acidified with 5 ml of 0.02 N Sulphuric acid and absorbance was measured in the spectrophotometer at 470 nm wavelength. A standard niacin solution was prepared and diluted. Ten mililitres (10 ml) of the solution was analyzed as discussed above. The readings were with reagent blank at zero. The vitamin B_3 was calculated using the formular given as follows:

$$\text{Niacin (mg/100g)} = \frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{Vf}{Va} \text{ ....Eqn 3.8}$$

Where:
W = weight of sample analysed
AU = Absorbance of the test sample.
AS = Absorbance of standard vitamin B3 solution
VF = Total Volume of filtrate
Va = Volume of filtrate analysed
C = Concentration of standard (mg/ml)
D = Dilution factor where applicable.

2.5.4 Determination of Pro-vitamin A (Carotenoid)

This was done using the method described by Delia *et al.* (2004) [12]. Ten grams (10g) of sample was weighed and crushed in mortar. Thirty milliliters (30ml) of cold acetone was used to extract the carotenoid. It was filtered by suction through a sintered glass funnel. The mortar, pestle, funnel, and residue were washed with 50 ml acetone through the funnel to the suction flask. About 20 ml of petroleum ether (40-60 ^{0}C) was poured into 500 ml separating funnel and each of the filtrate above was poured in slowly. Distilled water was introduced using wash bottles to the lining of the separating funnel. The lower aqueous phase was discarded. The washing was done 3-4 times to remove residual acetone. After this, the petroleum ether phase was collected in a 25ml volumetric flask which was made to pass through a funnel containing anhydrous Sodium sulphate to remove residual water (glass wool was plug to hold the sodium). It was made up to mark using petroleum ether and absorbance read at 450 nm using a spectrophotometer.

$$\text{Total carotenoid content } (\mu g/g) = \frac{A \times \text{volume (ml)} \times 10^4 \times D_f}{A1cm^{1\%} \times \text{weight of sample}} \text{ ....Eqn 3.10}$$

Where:
A = absorbance
Volume = absorbance coefficient of β-carotene in petroleum ether
D_f = dilution factor

2.5.5 Determination of ascorbic acid (Vitamin C)

This was determined based on the method described by Okwu and Josiah (2006) [10]. Ten grams (10 g) of the sample was extracted with 50 ml EDTA/TCA extracting solution for 1h, filtered through Whatman filter paper into a 50 ml volumetric flask and made up to the mark with the extracting solution. Twenty milliliters (20 ml) of the extract was pipetted into a 250 ml conical flask and 10 ml of 30% KI was added followed by addition of 50 ml of distilled water and 2 ml of 1% starch indicator. This was titrated against 0.01N $CuSO_4$ solution to a dark end point. The vitamin C content was calculated as follows:

$$\text{Vit.C (mg/100g)} = 0.88 \times \frac{100}{10} \times \frac{V_f}{20} \times \frac{T}{1} \text{ ....Eqn 3.11}$$

Where:
V_f = volume of extract
T = sample titre – blank titre

2.5.6 Determination of vitamin E (Tocopherols)

The vitamin E content of the sample was determined by the method described by Kirk and Sawyer (1998) [13]. A measured weight of the sample (2 g) was mixed with 10 ml of absolute alcohol (ethanol) and 20 ml of molar ethanolic sulphuric acid solution was added to it. The container was wrapped in aluminium foil to avoid direct light effect on the contents. It was boiled under reflux for 45 min and allowed to cool before 50 ml of distilled water was added to it. The mixture was transferred to a separating funnel and an additional 50 ml of distilled water was used to wash out the container into the funnel. One hundred and fifty mililitres (150 ml) of diethyl ether was added to it, mixed well and allowed to separate. The (ether layer) extract was collected and evaporated to dryness in a desicator over Sodium sulphate (anhydrous). The residue (vitamin E extract) was

redissolved in 10 ml of absolute ethanol and used for the analysis. One mililitre (1ml) aliquot of the extract was dispersed into the test tube. A standard vitamin E solution was prepared and diluted to a desired concentration. One mililitre (1ml) of the solution (vitamin E) and 1 ml of ethanol were dispersed into separate tubes to serve as standard and reagent blank respectively. About 5 ml of ethanol was added to each tube. A quantity of 1 ml of concentrated HNO_3 was also added drop wise with caution. The tubes were boiled at 90 ^{0}C in a water bath for 3 min, cooled with tap water and the absorbance measured in spectrophotometer at 470 nm wavelength. The reagent blank was used to calibrate the instrument to zero. The vitamin E content was calculated as follows:

$$E\ (mg/100g) = \frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{Vt}{Va} \times D \text{ ....Eqn 3.12}$$

Where:
W = weight of sample ashed
AU = Absorbance of test sample.
AS = Absorbance of standard solution
C = Concentration of standard solution
Vt = Total Volume of extract
Va = Volume of extract analyzed

2.6 Determination of mineral contents of African oil bean samples

The mineral contents of the test samples were determined by the dry ash extraction method as described by James (1995) [14]. The sample was burnt to ashes in a muffle furnace (Ash determination). The ash obtained was dissolved in 10 ml of dilute 2 M HCl solution and made up to volume with distilled water. It was filtered and the filtrate was used in specific analysis for different mineral elements.

2.6.1 Determination of phosphorus contents

Phosphorus content of the sample was determined by the Vanado-molydate colourmetric method described by James (1995) [14]. One gram (1g) of the extract from the sample was dispensed into a test tube. The same volume of standard phosphorus solution as well as water was added into different test tubes to serve as reagent blank and standard respectively. They were mixed with equal volume of the Vanado-molybdate color reagent, allowed to stand for 15 min and measured in Jenway Electronic spectrophotometer at a wavelength of 420 nm with a reagent blank at zero.The phosphorus content of the sample was given by the formula:

$$P\ (mg/100g) = 100 \times \frac{au}{w} \times \frac{c}{as} \times \frac{Vt}{Va} \text{ ....Eqn 3.13}$$

Where
W = weight of ashed sample
au = absorbance of test sample
as = absorbance of standard phosphorus solution
C = concentration of standard phosphorus solution
Vt =total volume of extract
Va = volume of extract analyzed

2.6.2 Determination of calcium and magnesium contents

Determination of Calcium and Magnesium contents of the sample extract was carried out using Versanate EDTA complexiometric titration described by Capentar and Hendricks (2003) [15]. Twenty millilitres (20 ml) of each extract was dispersed into a conical flask pinch doses of the masking agents (Potassium cyanide, Potassium ferocyanide, Hydroxylamine hydrochloride) were added to it. Then 20 ml of ammonia buffer was added to adjust the pH to 10.0. A pinch of the indicator Eriochrome black T was added and the mixture was shaken very well. It was titrated against 0.02N EDTA solution, until the colour changed from mauve to a permanent deep blue colour. This titration gave a reading for combined concentration of Ca and Mg ions. This occurred as a result of Ca^{2+} and Mg^{2+} forming complexes at pH 10.0 with EDTA. A second titration was conducted to determine Calcium alone. This was a repeat of the previous one with slight change, in that 10 % NaOH solution was used to raise the pH of the digest to 12.0 and then titrated with 0.02N EDTA using Selechrome dark blue as indicator in place of Eriochrome black T. At pH12.0, Ca^{2+} complexes with EDTA. A reagent blank was titrated to serve as control. The experiment was repeated andthe calcium and magnesium contents were calculated separately using the formula.

$$\%\ \text{Calcium or Magnesium} = \frac{100}{w} \times EW \times N \times \frac{Vt}{Va} \times T - B \text{ ....Eqn 3.14}$$

Where:
W = weight of sample analyzed
EW = equivalent weight
N = normality of EDTA
Vt = total volume of extract
Va = volume of extract titrated
T = titre value of the sample
B = titre value of blank

2.6.3 Determination of iron content

The Iron content was determined by Spectrophotometeric method described by James (1995) [14]. One millilitre (1 ml) of the sample was first digested with 20 ml of acid mixture that composed of 10 ml of concentrated HNO_3; 8 ml of Perchloric acid and 2 ml of H_2SO_4). The digest was diluted by making up to 100ml flask with distilled water.Two milliliters (2 ml) of the sample solution was pipetted into a conical flask,3 ml of buffer solution, 2 ml of hydroquinone solution and 2 ml of bipyridyl was then added.The absorbance reading was taken at a wavelength of 520 nm and the blank was used to zero the instrument. Also a standard solution was prepared by dissolving 3.152g of $Fe(NH_4)_2SO_4\ 6H_2O$ in water, two drops of 0.5 N HCl was added and diluted with 500 ml of distilled water. The Iron standard was further prepared at different concentration of 2 ppm to 10 ppm by diluting with distilled water, 3 ml of buffer solution, 2 ml of hydroquinone solution and 2 ml bipyridyl was also added. The absorbance reading was taken at 520 nm. The Iron content was calculated using the formula.

$$Fe\ (mg/100) = \frac{100}{W} \times \frac{AU}{AS} \times C \times \frac{Vt}{Va} \text{ ....Eqn 3.15}$$

Where,
W = Weight of sample analysed
AU = Absorbance of test sample.

AS = Absorbance of standard solution
C = Concentration of standard solution.
Vf = Total Volume of the filtrate
Va = Volume of filtrate analyzed

2.6.4 Determination of zinc content

Determination of zinc was done by the method of Pearson (1976). Five millilitres (5ml) of sample was pippeted into a test tube and 2 ml of citric acid solution was added and neutralized with ammonia. Five milliliters (5 ml) of dithizone solution was added and the solution was shaken and the lower layer discarded. The solution was allowed to stand for 30 min. Five milliliters (5 ml) of dilute dithizone was added and shaken. The absorbance of the dithizone layer was read at the wavelength of 532 nm.

$$Zn\ (mg/100) = \frac{100 \times AU \times C}{W \quad AS} \times \frac{Vt}{Va} \text{ ....Eqn 3.16}$$

Where,
W = weight of sample ashed, AU =Absorbance of test sample.
AS = Absorbance of standard solution
C = Concentration of standard phosphorus solution.
Vt = Total Volume of extract
Va = Volume of extract analyse

3.0 Results and Discussions

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

Results of pH, total titratable acidity, time and temperature of microbial inoculation. Figures 2a,b, and c show the values 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 24 h at temperature of 36.5 ^{0}C and pH of 6.30. Total titratable acidity (TTA) (g/ml) decreased with increase in pH. Onukwo (1992) and Aderibigbe and Adebayo (2002) reported the same increase in pH with decrease in TTA during fermentation of protein-rich oil seeds. An increase in temperature was observed with decrease in the period of fermentation. This may be attributed to the exothermic reaction displayed by the fermenting organisms.

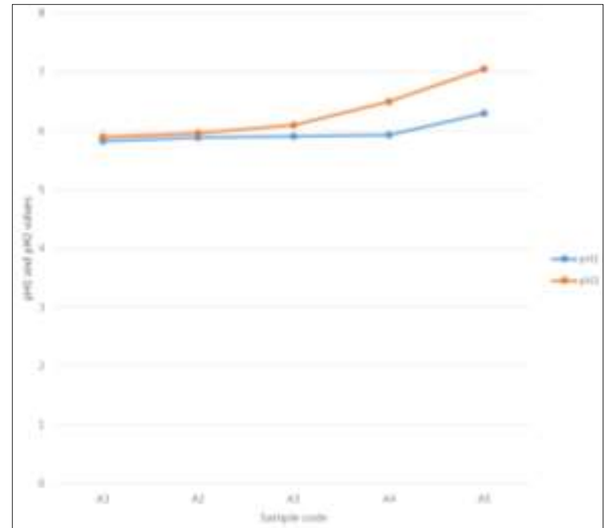

Fig 2a: Values for the initial and final pH for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

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*)

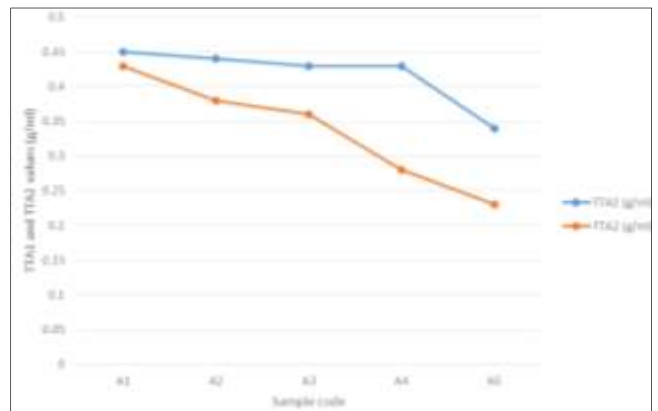

Fig 2b: Values for the initial and final TTA for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis*

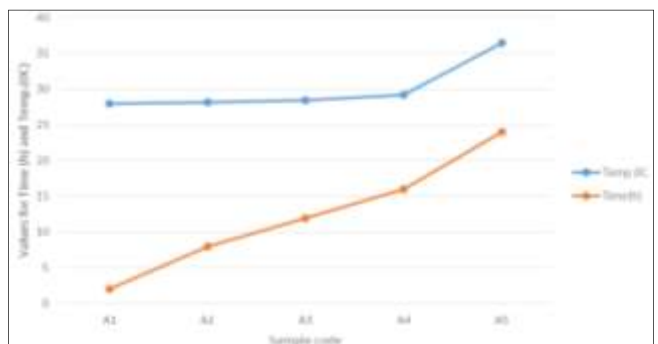

Fig 2c: Values for temp.^{0}C and fermentation times for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

3.2 Organoleptic properties of african oil bean samples fermented with *B. subtilis* as starter and *L. fermentum* as adjunct

Table 3.2 shows the organoleptic properties of African Oil Bean samples fermented with *B. subtilis* as starter and *L. fermentum* as adjunct. Samples A1 through A4 were not chosen as the best options for the use of *L. fermentum* as adjunct culture during the initial fermentation of African Oil Bean with *B. subtilis* because of the nature of the African Oil Bean samples obtained. This may be attributed to the conditions under which the adjunct culture was added for acid fermenter and alkaline fermenter cannot work under the same condition. Moreso, the fermentation process could still be another contributing factor for *B. subtilis* grows very well under aerobic condition although it can as well grow under anaerobic condition. However sample A5 was chosen based on some organoleptic parameters (appearance, aroma, taste and texture) analysed.

Table 3.2: 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

3.3 Vitamin composition (mg/100g) of African oil bean samples

Significant differences ($p<0.05$) existed between sample E (cooked unfermented African Oil Bean sample) and fermented samples as shown in table 3.3. This could be attributed to the effect of fermentation on water soluble vitamins during processing especially thiamin. In terms of vitamin $B_{2,}$ No significant difference ($p>0.05$) existed between samples A and C (African oil bean fermented *B. subtilis* and African Oil Bean sample fermented with *B. subtilis* and *Lactobacillus fermentum*) likewise samples B and D (African Oil Bean sample fermented with *Lactobacillus fermentum* and African Oil Bean sample fermented traditionally). This may be attributed to the type and nature of the organisms involved in the fermentation process. Sample F (Raw African oil bean sample) had the highest vitamin B_2 content (0.071mg/100g)**.** Significant difference ($p<0.05$) existed between samples B and C (African oil bean sample fermented with *Lactobacillus fermentum* and African Oil Bean sample fermented with *B. subtilis* and *Lactobacillus fermentum).* This may be due to the organism that initiated the fermentation process. No significant difference ($p>0.05$) was observed between samples C and D (African Oil Bean sample fermented with *B.subtilis* and *Lactobacillus fermentum* and African Oil Bean fermented traditionally) but samples A,B,C and D (African Oil Bean sample fermented with *B. subtilis*, African Oil Bean sample fermented with *Lactobacillus fermentum,* African Oil Bean sample fermented with *B. subtilis* and *Lactobacillus fermentum* and African Oil Bean sample fermented traditionally) were significantly different ($p<0.05$) from sample E(cooked unfermented sample).This may be attributed to modification of nutrients especially Vitamin A in African Oil Bean as a result of fermentation. Their values ranged from 0.482 to 6.700 mg/100g. The vitamin E content values ranged from 2.150 to 9.385 mg/100g. Vitamin C aids in the absorption of Iron from the intestines, in the healing of wounds and in teeth and bone formation (Okaka and Okaka, 2001) [18]. Sample F (Raw African oil bean sample) had the highest value (18.290 mg/100g) while sample E had the least value (4.220 mg/100g).

Table 3.3: Vitamin composition (mg/100g) of African oil bean samples

Sample code	Parameters					
	B1	B2	B3	A	E	C
A	$0.012^{c}\pm0.00$	$0.042^{bc}\pm0.00$	$0.312^{c}\pm0.01$	$4.350^{d}\pm0.00$	$4.270^{d}\pm0.11$	$12.210^{d}\pm0.02$
B	$0.021^{ab}\pm0.00$	$0.034^{d}\pm0.00$	$0.304^{cd}\pm0.00$	$5.140^{b}\pm0.11$	$5.180^{b}\pm0.03$	$12.930^{b}\pm0.16$
C	$0.017^{bc}\pm0.00$	$0.046^{b}\pm0.02$	$0.327^{b}\pm0.04$	$0.482^{f}\pm0.00$	$4.750^{c}\pm0.00$	$12.670^{c}\pm0.01$
D	$0.016^{bc}\pm0.00$	$0.035^{cd}\pm0.00$	$0.294^{d}\pm0.01$	$4.800^{c}\pm0.00$	$2.150^{e}\pm0.00$	$11.430^{e}\pm0.39$
E	$0.010^{c}\pm0.00$	$0.016^{e}\pm0.01$	$0.112^{e}\pm0.01$	$2.950^{e}\pm0.04$	$2.150^{e}\pm0.00$	$4.220^{f}\pm0.85$
F	$0.028^{a}\pm0.00$	$0.071^{a}\pm0.00$	$0..423^{a}\pm0.06$	$8.670^{a}\pm0.04$	$9.385^{a}\pm0.09$	$18.290^{a}\pm0.09$

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, E - Cooked unfermented African Oil Bean sample, F - Raw African oil bean sample

3.4 Mineral composition (mg/100g) of African oil bean samples

Table 3.4 shows the mineral compositions of different African Oil Bean samples. Calcium helps in the development of bones, teeth and in blood clothing. Sample A (African Oil Bean fermented with *Bacillus subtilis*) rated highest (256.47 mg/100g) followed by sample C (African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillus fermentum)* (252.35 mg/100g) and sample F (Raw African Oil Bean sample) had the least value (212.59 mg/100g). These values are higher than the value obtained by Nwachukwu *et al.* (2018) [17] who reported a value of 92.11mg/100g after fermentation of African Oil Bean for a period of 72h.

However, the value of 318.28mg/100g reported by Enujiugha and Akanbi (2005) [5] during fermentation of African Oil Bean for a period of 72 h is higher than the values obtained in this study. Magnesium plays an important role in the maintenance of muscle functions. Significant differences ($p<0.05$) were observed between fermented and unfermented samples. This is attributed to the effect of fermentation on African Oil Bean samples as it brings about

modification in nutrient bioavailability and digestibility as reported by Enujiugha and Akanbi (2005) [5]. Phosphorus helps to keep the body fluid composition balanced. Their values ranged from 186.86 to 232.10 mg/100g. These results are not in agreement with what Okechukwu *et al*. (2012) [19] reported on the effects of fermentation on the phosphorus contents of African Oil Bean fermented at intervals for a period of 7 days. Iron, the red substance in the blood helps in transferring oxygen from the lungs to the brain, muscles and other parts of the body (Okaka and Okaka, 2001) [18]. Sample B (African Oil Bean sample fermented with *Lactobacillus fermentum)* rated highest (10.58 mg/100g) while sample E (Cooked Unfermented African Oil Bean sample) rated lowest (8.43mg/100g). Zinc is essential for the production of insulin in the body (Okaka an. The Zinc content values ranged from 5.33 to 9.85 mg/100g.

Table 3.4: Mineral composition (mg/100g) of African Oil Bean samples

Sample code	Parameters				
	Ca	Mg	P	Fe	Zn
A	$256.47^{a}\pm0.00$	$216.66^{b}\pm0.00$	$229.32^{c}\pm0.03$	$10.18^{c}\pm0.00$	$9.60^{b}\pm0.00$
B	$248.46^{c}\pm0.00$	$212.59^{c}\pm0.00$	$234.74^{a}\pm0.06$	$10.58^{a}\pm0.00$	$9.85^{a}\pm0.00$
C	$252.32^{b}\pm0.03$	$219.39^{a}\pm0.03$	$232.10^{b}\pm0.00$	$10.41^{b}\pm0.01$	$9.40^{c}\pm0.11$
D	$241.52^{d}\pm0.00$	$204.24^{d}\pm0.06$	$222.30^{d}\pm0.00$	$9.73^{d}\pm0.04$	$9.31^{c}\pm0.14$
E	$224.15^{e}\pm0.00$	$176.40^{f}\pm0.28$	$186.86^{f}\pm 0.00$	$8.43^{e}\pm0.04$	$7.06^{d}\pm0.00$
F	$212.59^{f}\pm0.00$	$192.65^{e}\pm0.42$	$210.75^{e}\pm 0.00$	$9.25^{e}\pm0.00$	$5.33^{e}\pm0.11$

Means not followed by the same superscript in the same column are significantly different ($p<0.05$).

4. Conclusion

The study revealed that *L. fermentum* can serve as a good adjunct culture to *B.subtilis* starter for fermentation of African oil bean. The vitamin and mineral contents of fermented African oil bean samples increased as a result of fermentation.

5. References

1. Subramaniyam, R. and R. Vimala. Solid state and submerged fermentation for the production of bioactive substances: A comparative study. Int. J. Sci. Nat. 2012; 3:480-486.
2. FAO. Food agriculture organization fermented fruits and vegetables, A global perspective, 1998, Bulletin No.134, FAO Agricultural Services, Rome.
3. Aidoo KE. Application of biotechnology to indigenous fermented foods. Proc Technol. Dev. Ctries. 1994; 12(2/3):83-93.
4. Keay, R.W.J. *Trees of Nigeria*. Clarendon Press, Oxford, 1989, p259.
5. Enujiugha VN, Akanbi CT. Compositional changes in African oil bean (*Pentaclethra macrophylla* Benth) seeds during thermal processing. Pakistan Journal of Nutrition. 2005; 4:27-31.
6. Norman FH, Odunfa SA, Charl-Ho L, Quintero-Ramirez R, Wacher-Radarte C, Lorence-Quinones A. Fermented food cereals:A global perspective. Food and Agriculture Organisation, Rome, 1999. ISBN 92-5-104296-9.
7. Achinewhu SC, Ryley J. Effect of fermentation on the thiamin, riboflavin and niacin contents of melon seeds (*Citrulum vulgaris*) and African Oil Bean Seed (*Pentaclethra macrophylla)* Biotechnology 19:733-736. Agroforestry Systems. 1986; 5(2):153-168.
8. Enujiugha VN, Olagunde TV. Comparative study on the nutritional characteristics of raw fermented and roasted African oil bean (*Pentaclethra macrophylla* Benth) seeds. Rivista Italiana delle Sostanze Grasse, 2001; 78:247-250.
9. Obeta JAN. A note on the Microorganisms associated with the fermentation of Seeds of the African Oil Bean Tree (*Pentaclethra macrophylla*). Journal of Applied Bacteriology. 1983; 54:433-435.
10. Okwu DE, Josiah C. Evaluation of the Chemical composition of two Nigerian Plants. African Journal of Biotechnology. 2006; 5(4):357-361.
11. AOAC. Official Methods of Analysis. 2006; 18th Edn., Washington, DC., USA.
12. Delia B, Rodriguez-Amaya DB, Kimura M. *Harvest Plus Handbook for Carotenoid Analysis*, 2004. Technical Monograph Series 2.
13. Kirk RS, Sawyer R. *Pearson's composition and composition analysis of foods*. 9th edn. Churchill living Stone. 21998. Edingburgh, 1998, p615-616.
14. James CS. *Analytical chemistry of food.* Chapman and Hall, London, 1995, 64–65.
15. Carpenter S, Hendricks P. In: Pearson Laboratory techniques in food analysis. Butter worth and co. publishing Ltd., 2003, p10-17.
16. Pearson D. Chemical Analysis of Foods. 7th Edn., Church Hill Livingstone, London, UK., 1976, p72-73.
17. Nwachukwu CD, Enyoh EC, Enyoh CE, Amaobi CE. Effect of fermentation on the proximate and mineral composition of fermented African Oil Bean seed “Ugba”, 2018.
18. Okaka JC, Okaka ANC *Foods:Composition, Spoilage Shelf-Life Extension*. Food Commodity series Number 1.OcJANCO Academic Publishers. Independent layout, Enugu. 2001, p230-233.
19. Okechukwu RI, Ewelike N, Ukaoma AA, Emejulu AA, Azuwike CO. Changes in the nutrient composition of the African Oil Bean meal “ugba” (*Pentaclethra macrophylla* Benth) subjected to solid state natural fermentation. Journal of Applied Biosciences. 2012; 51:3591–3595.
20. Harold, Eddleman, Ph. D (February 1998). "Making Bacteria Media from Potato". Indiana Biolab. disknet.com. Retrieved, 2011.