World Journal of Innovative Research (WJIR)
ISSN: 2454-8236, Volume-8, Issue-6, June 2020 Pages 132-138

Effect of Starter and Adjunct Cultures on Amino Acid Profile of Fermented African Oil Bean Samples

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***Abstract*— Effect of starter and adjunct cultures on amino acid profile of fermented African oil bean samples was studied. African Oil Bean was processed and subjected to chance fermentation. The fermentation process lasted for a period of 96 (h) . 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 were investigated. The amino acid profiles of fermented African oil bean samples were investigated. All the fermented African Oil Bean samples analysed contained all the essential amino acid.There were reduction in the values of some amino acids like methionine, cystine and trptophan while others like glutamic acid, glycine, aspartic acid, leucine, arginine, alanine, valine, and phenylalanine increased. Starter culture fermented African oil bean samples had high lysine content than the samples fermented traditionally likewise cooked unfermented samples.**

***Index Terms*— Starter and adjunct culture,amino acid, African oil bean. .**

I. INTRODUCTION

Proteins are made up of building blocks called amino acids. Protein from food comes from plant and animal sources such as meat and fish, eggs, dairy products, seeds and nuts, and legumes like beans and lentils. There are about twenty different amino acids that link together in different combinations. The body uses them to make new proteins, such as muscle and bone, and other compounds such as enzymes and hormones. Some amino acids can be made by the body. There are eleven of these and they are known as non-essential amino acids. There are nine amino acids that the body cannot make, and they are known as essential aminoacids.

African Oil Bean belongs to the leguminosae family, tropical tree crop which is found in the southern rain forest zone of West Africa (Keay, 1989). The seed is an edible product and a source of oil, hence the name, "African Oil Bean" (Ugba). African Oil Bean undergoes fermentation which makes it 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. Different methods are adopted by different people resulting in product with short duration period. Moreso, it undergoes fermentation by diverse microorganisms (Enujiugha and Akanbi, 2005).

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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). 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).

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; Rolle and Satin, 2002). Fermentation activities when combined with a variety of other traditional and domestic activities can improve livelihoods of women, and other less priviledge people who with appropriate training can boast up their nutritional status through income generation (FAO, 1999)

Statement of problem

Essential amino acids can not be synthesized by the body rather are gotten from diet. African oil bean contains essential amino acid but can not be consumed in its raw state. A lot of researchers including Enujiugha and Akanbi (2005), Eze *et al.*,2014 etc reported fermentation of African oil bean traditionally by diverse microorganisms and in a controlled manner. However, African oil bean has been fermented with*Bacillus subtilis* as starter and *Lactobacillus fermentum* as adjunct (Nwanagba *et al*., 2020) although not with respect to evaluation of amino acid profile of African oilbean fermented with the above starter and adjunct cultures. This study aimed at evaluating the effect of starter and adjunct cultures on amino acid profile of fermented African oil bean samples

II. 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* 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.

2.2 METHODS

2.2.1. 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 in the following manner: The colony appearance 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; and 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 (^{0}C), 45 (^{0}C) as well as growth in potato dextrose agar (PDA) at room temperature (30±2^{0}C). 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.

2.2.2 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 inserted in the bore hole of the fermenting substrate at different periods respectively (2, 8, 12, 16 and 24 h). 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 (see results section).

2.2.3. 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 Obetta (1983). 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.

2.2.4 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.

2.2.5Preparation 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.

2.3 PRELIMINARY INVESTIGATION ON THE USE OF *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. subtilisat* 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 0.1 (N) 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 C24 (h) through C96 (h) for the initial fermentation period of 24 (h) 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 E0 (h) (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 24 (h) 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 amino acid profile

The amino acid profile in the known sample was determined using the method described by Benitez (1989). The known sample was dried to constant weight, defatted, hydrolyzed, evaporated in a rotary evaporator and loaded into the Applied Biosystems PTH Amino Acid Analyzer.

(i) Defatting sample:

The sample was defatted using chloroform/methanol mixture of ratio 2:1. About 300 (mg) of the sample was put in extraction thimble and extracted for 15 (h) in soxhlet extraction apparatus (AOAC, 2006).

(ii) Determination of nitrogen:

A small amount (0.115 mg) of ground sample was weighed, wrapped in Whitman filter paper (No.1) and put in the Kjeldhal digestion flask. Concentrated sulphuric acid (10 ml) was added. Catalyst mixture (0.5 g) containing sodium sulphate (Na_2SO_4), copper sulphate ($CuSO_4$) and selenium oxide (SeO_2) in the ratio of 10:5:1 was added into the flask to facilitate digestion. Four pieces of anti-bumping granules were added.

The flask was then put in Kjeldhal digestion apparatus for 3 (h) until the liquid turned light green. The digested sample was cooled and diluted with distilled water to 100 (ml) in standard volumetric flask. Ten milliliters aliquot of the diluted solution with 10 (ml) of 45 (%) sodium hydroxide was put into the Markham distillation apparatus and distilled into 10 (ml) of 2 (%) boric acid containing 4 drops of bromocresol green/methyl red indicator until about 70 (ml) of distillate was collected. The distillate was then titrated with standardize 0.01 (N) hydrochloric acid to grey coloured end point.

$$\text{Percentage Nitrogen} = \frac{(a-b) \times 0.01 \times 14 \times V \times 100}{W \times C}$$

Where:

a. = Titre value of the digested sample
b. = Titre value of blank sample
v. = Volume after dilution (100 ml)
W. = Weight of dried sample (mg)
C. = Aliquot of the sample used (10 ml)
14. = Nitrogen constant in mg.

(iii) Hydrolysis of the sample

A known weight of the defatted sample was weighed into glass ampoule. Seven milliliters (7 ml) of 6 (N) HCl was added and oxygen was expelled by passing nitrogen into the ampoule (this is to avoid possible oxidation of some amino acids during hydrolysis e.g methionine and cystine).The glass ampoule was then sealed with Bunsen burner flame and put in an oven preset at 105 (^{0}C)± 5 (^{0}C) for 22 (h). The ampoule was allowed to cool before broken open at the tip and the content was filtered to remove the humins. The filtrate was then evaporated to dryness using rotary evaporator. The residue was dissolved with 5 (ml) acetate buffer (pH 2.0) and stored in plastic specimen bottles, which were kept in the freezer prior to analysis. Sixty microlitre (60 µl) of the sample was dispensed into the cartridge of the analyzer designed to separate and analyze free acidic, neutral and basic amino acids of the hydrolysate. An integrator attached to the Analyzer calculated the peak area proportional to the concentration of each of the amino acids.

2.6 STATISTICAL ANALYSIS

The data obtained from duplicate analyses of amino acid profiles of fermented African oil bean samples were subjected to analysis of variance of a completely randomized design (C.R.D) using the SPSS procedure version 22 for personal computers while treatment mean were separated using Duncan's multiple range test at significant difference of (95%) confidence level ($p<0.05$).

III. RESULTS AND DISCUSSION

3.1. Results of preliminary experiments to determine the best option for the use of *L.fermentum* as an adjunct starter to B. subtilis

3.1.1ORGANOLEPTIC 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. 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 since the acid fermenter (*L. fermentum*) and alkaline fermenter *(B. subtilis*) cannot work under the same condition. More so, the fermentation process could still be another contributing factor because *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) analyzed.

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*)

3.1.2 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 24 (h) at a temperature of 36.5 (^{0}C) and pH of 6.30. Total titratable acidity (TTA) (g/ml) decreased with increase in pH. 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.

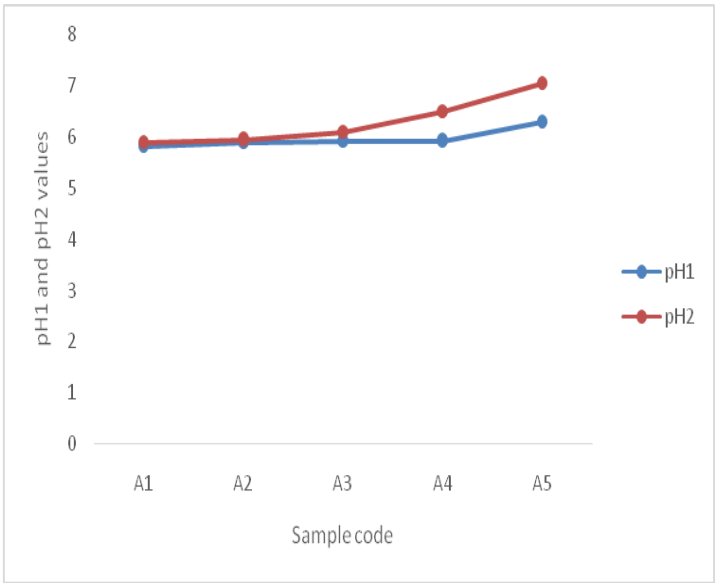

Fig.1a: Initial (pH1) and final pH plots for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

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*).

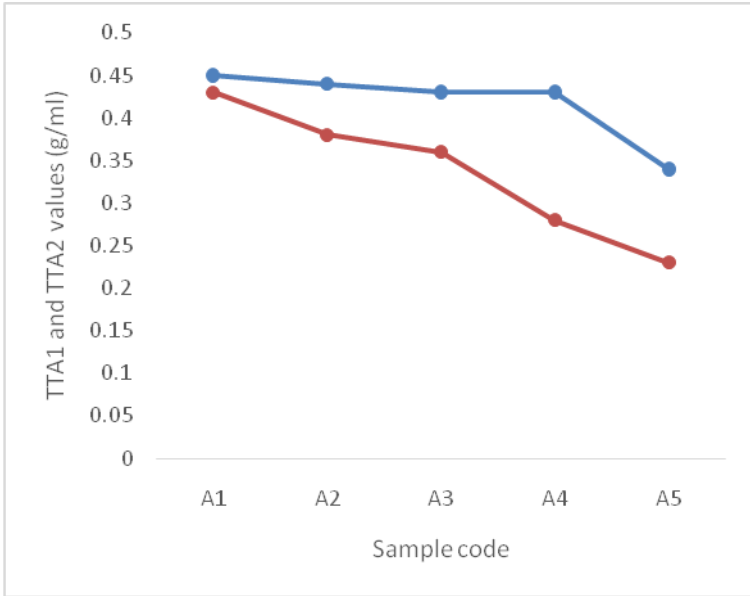

Fig.1b: Plot of the initial and final TTA for the different

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*)

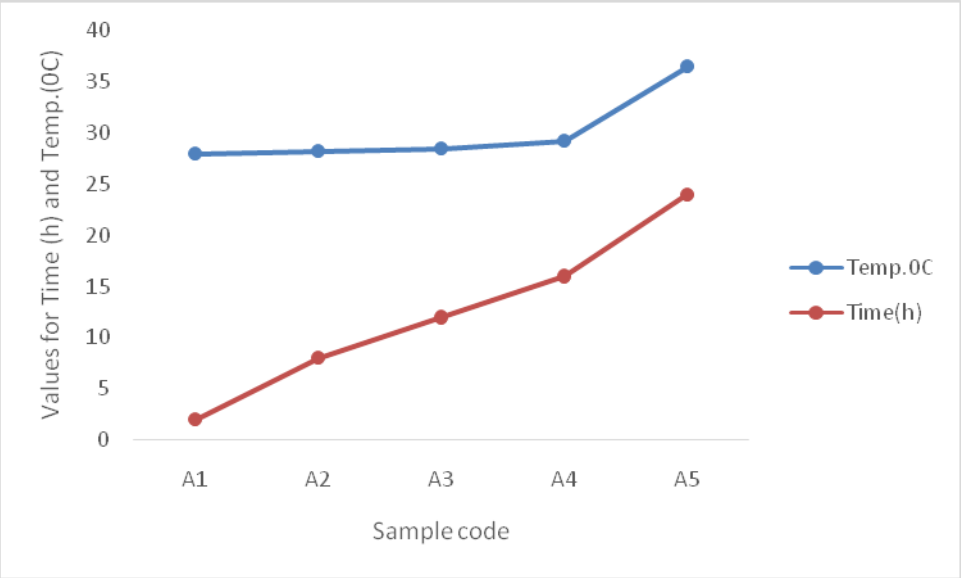

Fig.1c: Plot of values for temperature (^{0}C) and fermentation times for the different samples used for evaluation of the best option for addition of *Lactobacillus fermentum* after the initial fermentation with *Bacillus subtilis.*

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*)

Table 2 shows the amino acid profile of African Oil Bean samples. All the fermented African Oil Bean samples analysed contained all the essential amino acid. This is attributed to the fact that African Oil Bean is rich in protein. This result is in agreement with what was reported by Enujiugha and Agbede, 2000 and Ogbo 2007. There were reduction in the values of some amino acids like methionine, cystine and trptophan. The reason may be that some of the free amino acids are utilized in building up the protein sector of the substrates during the fermentation process. The increase observed in some like glutamic acid, glycine, aspartic acid, leucine, arginine, alanine, valine, and phenylalanine was due to biosynthesis of amino acid, reaction between ammonia and a 5-C intermediate compound metabolism which are converted to an amino acid which in turn is useful in the formation of other amino acids (Nester *et.al.*, 1973). Starter culture fermented African oil bean samples had high lysine content than the samples fermented traditionally likewise cooked unfermented samples. This is in-line with Tosaka *et al*. (1983), who reported that the production of high content of lysine was as a result of substrate fermentation with *Candida glutamicum* and *Escherichia coli*.

Table 2: Amino acid Composition of African Oil Bean samples

Parameters	Sample codes					
	A	B	C	D	E	F
Leucine	7.29^{c} ± 0.01	7.99^{a} ± 0.00	5.95^{f} ± 0.01	7.00^{d} ± 0.00	7.50^{b} ± 0.00	6.33^{e} ± 0.00
Lysine	4.03^{c} ± 0.01	4.69^{a} ± 0.01	3.92^{d} ± 0.01	3.55^{e} ± 0.01	4.19^{b} ± 0.01	3.52^{e} ± 0.00
Isoleucine	3.34^{a} ± 0.01	3.53^{a} ± 0.00	3.42^{a} ± 0.01	3.27^{a} ± 0.00	3.90^{a} ± 0.74	3.01^{a} ± 0.01
Phenylalanine	4.71^{b} ± 0.01	4.43^{a} ± 0.00	4.34^{a} ± 0.01	3.99^{c} ± 0.01	3.72^{d} ± 0.23	3.90^{c} ± 0.28
Tryptophan	0.89^{d} ± 0.01	1.10^{a} ± 0.00	1.02^{b} ± 0.01	0.84^{e} ± 0.00	0.99^{c} ± 0.01	0.79^{d} ± 0.00
Valine	3.89^{a} ± 0.01	4.00^{a} ± 0.01	3.62^{b} ± 0.01	3.45^{c} ± 0.01	3.92^{a} ± 0.03	3.33^{c} ± 0.01
Methionine	0.99^{c} ± 0.00	1.55^{a} ± 0.01	0.96^{c} ± 0.00	0.91^{d} ± 0.01	1.23^{b} ± 0.01	0.91^{d} ± 0.01
Proline	3.14^{b} ± 0.03	3.25^{a} ± 0.00	2.94^{d} ± 0.00	3.25^{a} ± 0.00	2.84^{e} ± 0.01	3.04^{c} ± 0.01
Arginine	5.07^{b} ± 0.01	6.28^{a} ± 0.01	4.99^{c} ± 0.01	4.81^{d} ± 0.00	3.16^{f} ± 0.03	4.56^{e} ± 0.01
Tyrosine	3.09^{b} ± 0.00	3.44^{a} ± 0.01	2.75^{d} ± 0.01	2.92^{c} ± 0.01	3.09^{b} ± 0.01	2.46^{e} ± 0.00
Histidine	2.20^{a} ± 0.01	2.23^{a} ± 0.01	2.11^{b} ± 0.01	1.95^{c} ± 0.01	2.14^{b} ± 0.01	1.85^{d} ± 0.01
Cystine	0.91^{d} ± 0.01	1.09^{a} ± 0.00	0.72^{f} ± 0.00	0.84^{e} ± 0.00	0.97^{c} ± 0.01	1.02^{b} ± 0.01
Alanine	3.94^{c} ± 0.01	4.43^{a} ± 0.01	4.17^{b} ± 0.01	3.90^{d} ± 0.00	3.94^{c} ± 0.01	3.83^{e} ± 0.01
Glutamic acid	10.90^{d} ± 0.00	12.26^{a} ± 0.03	11.58^{b} ± 0.01	10.90^{d} ± 0.01	11.20^{c} ± 0.28	10.82^{d} ± 0.00
Glycine	3.32^{d} ± 0.03	3.82^{b} ± 0.00	3.65^{c} ± 0.00	3.06^{f} ± 0.01	3.96^{a} ± 0.00	3.16^{d} ± 0.01
Threonine	3.24^{b} ± 0.00	3.27^{b} ± 0.03	2.99^{c} ± 0.01	3.11^{d} ± 0.01	3.33^{a} ± 0.00	2.49^{d} ± 0.00
Serine	3.29^{c} ± 0.01	3.59^{a} ± 0.01	3.45^{b} ± 0.01	3.24^{d} ± 0.01	2.94^{e} ± 0.01	3.24^{d} ± 0.03
Aspartic acid	7.75^{d} ± 0.01	8.34^{b} ± 0.03	8.00^{c} ± 0.00	7.75^{d} ± 0.04	8.62^{a} ± 0.01	7.60^{e} ± 0.03

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

A African Oil Bean sample fermented with *Bacillus subtilis*

B African Oil Bean sample fermented with *Lactobacillusfermentum*

C African Oil Bean sample fermented with *Bacillus subtilis* and *Lactobacillusfermentum*

D African Oil Bean sample fermented traditionally, E Cooked unfermented African Oil Bean sample. F Raw African Oil Bean sample.

IV. CONCLUSION

The study revealed that *L. fermentum* can serve as a good adjunct culture to *B. subtilis* starter for fermentation of African Oil Bean. Some amino acids contents such as valine, aspartic acid,alanine,arginine etc of African Oil Bean increased while others such as leucine, threonine, methionine, cystine etc decreased as a result of fermentation.

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