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# EFFECT OF *BACILLUS SUBTILIS* CONCENTRATION AND FERMENTATION TIME ON THE QUALITY OF AFRICAN LOCUST BEAN CONDIMENT

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**ABSTRACT**

Fermented locust bean condiment in Nigeria has continued to attract low market value. This may partly be due to inconsistent quality of the product. In order to improve the quality of the condiment, there is need to explore standardization of the production process through use of starter culture such as *Bacillus subtilis*. Six samples were developed and were fermented at 35 °C in the laboratory using *Bacillus subtilis* concentrations of 1.0 mL and 1.5 mL at fermentation time(s) of 24 h, 48 h and 72 h respectively. The pH, microbiological, chemical and sensory attributes of the fermented locust bean condiment samples were analysed using standard methods and a ten member semi trained panelists on a 3 point hedonic scale. As the dose concentration of *Bacillus subtilis* increased with fermentation time, the pH and microbial load showed a corresponding increase; except at fermentation time of 24 h where the total viable counts of the fermented locust bean were insignificant to be counted. The peroxide, FFA and TBA values of the fermented locust bean condiment samples showed increases, while protein exhibited a decline in content. *Bacillus subtilis* concentration of 1.0 mL and a fermentation time of 48 h produced a condiment with higher degree of consumer acceptability with no unpleasant odour. The use of *Bacillus subtilis* concentration was found to be concentration and time dependent. Excessive fermentation activities of *Bacillus subtilis* were undesirable at 72 h as this gave rise to low quality locust bean condiment with unpleasant odour.

**Keywords:** *Bacillus subtilis*, concentration, fermentation time, locust bean condiment

## INTRODUCTION

Fermentation is the chemical transformation of organic substrate into simpler compounds by the action of enzymes which are produced by microorganisms such as moulds, yeasts and bacteria **(Iwuoha and Eke, 1996; Omodara and Olowomofe, 2015)**. It is recognised as one of the age-long methods of food preservation across the globe. Fermented foods are essential components of the diet in a number of developing countries and are consumed either as main dishes or as condiments **(Ojinnaka *et al*., 2013**). By the process of using beneficial microorganisms, plant and animal sources can be transformed into fermented food products with improved quality attributes. Fermentation of food materials is significant in many ways; it reduces toxicity, improves palatability, impacts desirable flavor in foods **(Chelule *et al*., 2010; Omodara and Olowomofe, 2015)**, enhances the nutrient content of foods through biosynthesis of vitamins, essential amino acids and proteins, by improving protein and fibre digestibility, by enhancing micronutrients bio-availability, and by degrading antinutritional factors **(Ojinnaka *et al*., 2013)** and foods produced in this way have a reduced risk of contamination when enriched in antimi- crobial end-products, such as organic acids, ethanol, and bacteriocins **(Marco *et al.*, 2017)**.
Food condiments or spices are strong smelling, sharp tasting substances usually used to improve or adjust the flavour **(Odebunmi *et al*., 2010**). Reports from literature has established that there are nutrient-rich crops in Nigeria which are fermented and used as foods or as food condiments **(Achinewhu, 1983a, 1983b; Akinrele, 1970; Ekundayo, 1977; Kuboye, 1985; Odunfa and Oyeyiola, 1985; Ogundiwin, 1978a; Onyekwere, 1977; Pierson *et al*., 1986; Tehinse and Ogundiwin, 1978; Uzogara *et al*., 1990)**. Fermented food condiments give pleasant aroma to soups, sauces and other prepared dishes worldwide, especially in most African countries and India where protein calorie malnutrition is a major problem **(Ajayi, 2014)**. Oil seeds such as African locust bean, melon seed, castor oil seed, mesquite bean and soybean are fermented to give condiments (**Ajayi, 2014**).The African locust bean condiment is a popular fermented soup condiment in Western and Central African regions. Locust bean tree is found throughout the savannah lands of North Central Nigeria covering Benue, Kaduna, Kwara, Kogi, Nassarawa and Plateau States **(Simonyan, 2012).** *Parkia biglobosa* as it is also called, grows mainly in the wild state, most of its trees have not been domesticated and it takes a long time for the trees to fruit **(Dunsin *et al*., 2014)**. It is known by different names among the ethnicities in North-Central Nigeria; however, the most popular names are *Dawadawa* or *Dorowa* (Hausa-speaking people), *Igba* (Yoruba), *Ogirili* (Igbo) and *Nune* (Tiv) **(Tee *et al*., 2009)**. In many parts of Nigeria, African Locust Beans (ALB) is a popular delicacy especially as there is a growing interest in natural food ingredients as additives in consumer diets **(Ifeanyieze *et al*., 2016)**.The most important use of African locust bean is found in its seed, which is a grain legume, although it has other food and non - food uses, especially the seeds which serves as a source of useful ingredients for consumption **(Mohammed *et al*., 2017)**. Not only does it have a good repository of protein, especially amino acids such as lysine which is limiting in cereals, it also has B-Vitamins, in particular, riboflavin and fatty acids in good amounts. African locust beans have shown promise in boosting cellular immunity in immune compromised persons, fermented locust bean seed is used in controlling diabetes and cholesterol level, helps in the management of diarrhea and heart disease and as an antidote to snake bite, it also serves as foods supplement to malnutrition, helps to reduce blood sugar and management of bacterial infections **(Mohammed *et al*., 2017)**.
Published studies on the microbiology of the fermentation of locust beans seeds have identified *Bacillus spp* as the main microorganism responsible for fermentation of locust beans and the predominant species is *Bacillus subtilis*, but other species such as *B. pumilus*, *B. lichenformis* has been identified **(Odunfa, 1985)**. The traditional fermentation method which involves development of various lactic acid producing bacteria is a spontaneous process. Unfortunately, this widely practiced method in sub-Saharan Africa has always had its attendant challenge of inconsistent quality of the product and other related issues. In Africa, majority of the fermented foods are produced at household level and hygiene is a major concern **(Olasupo *et al*., 2002; Gadaga *et al*., 2008)**. Therefore, if the hygienic condition of the process is compromised, then the problem of occurrence and growth of pathogens may most likely occur in most of these fermented locust bean condiment.
So far, locust bean condiment has continued to attract low market value. There is therefore, need to produce locust bean condiment with higher market value through standardization of the production process. The use of starter cultures has generally been recognized as one major way of ensuring product consistency and to a reasonable extent eliminates the problem of food-borne pathogens **(Okorie and Olasupo, 2013)**. The dominance of *Bacillus subtilis* can be associated to its ability to produce antibiotics, which are active against other microorganisms that may likely want to partake in the fermentation process **(Campbell-Platt, 1980)**. Starter cultures offer a number of benefits such as: it guarantees product quality of fermented products, there is control of fermentation time, reduction of hygienic risk **(Gberikon and Agbulu, 2015)** and they increase safety by outcompeting undesirable microorganisms **(Laranjo *et al*., 2017**). The objective of our study was to evaluate the effect of *Bacillus subtilis* concentration and fermentation time on the quality of locust bean condiment.

## MATERIAL AND METHODS

### Collection of samples

Raw locust bean seeds were purchased from Gboko market, Gboko and *Bacillus subtilis* was obtained from Pathology and Microbiology laboratory of the Department of Veterinary Medicine, University of Agriculture, Makurdi; all in Benue state, North-Central Nigeria.

**Table 1** Experimental Design

| Concentration of *B. subilis* (in mL) | Fermentation period | | |
|---|---|---|---|
| | 24 h | 48 h | 72 h |
| 1.0 | A | C | E |
| 1.5 | B | D | F |

**Legend:** A = 24 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, B= 24 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, C= 48 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, D= 48 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, E= 72 h fermented locust beans at 1.0 mL concentration of *B. subtilis* and F= 72 h fermented locust beans at 1.5 mL concentration of *B. subtilis*.

### Preparation of fermented locust bean condiment

About 1400 g of locust bean seeds were prepared according to the method described by **Achi (2005)**. The locust beans were cleaned to remove stones and other unwanted materials. The seeds were cooked for 12 h in excess water until they are very soft and dehulled by hand separation. Boiling was carried out for 1 h, after which the seeds were drained and cooled to 40 $^{\circ}$C. *Bacillus subtilis* at concentration of 1.0 mL and 1.5 mL was used for inoculation and the cotyledon was incubated at 35 $^{\circ}$C. The fermented samples were analyzed for pH, Total viable count, peroxide value, free fatty acid, protein, thiobarbituric acid and sensory attributes respectively.

### Determination of pH

The pH of the sample was determined using sterile probes of the pH meter (Hanna Instruments, 8520) according to the standard method of **AOAC (2005)**.

### Microbial Analysis

### Total viable count

The method reported by **Adegoke (2004)** was adopted. About 9 mL of diluent (distilled water) was measured into culture tubes, arranged and labeled $10^{-1}$ to $10^{-4}$. 2.7 g of nutrient agar was weighed in a 250 mL conical flask and diluted to mark. Both the diluent and the nutrient agar were put into autoclaves and heated. Then 1 mL of sample was weighed and poured into the first tube and serial dilution prepared. About 0.1 mL of the dilution was transferred to sterile petri dishes and the cool nutrient agar poured and swirled very vigorously and gently. The petri dishes were allowed to solidify and transferred to an incubator at 37 ºC for 18 – 24 h. Total viable count (cfu/g) was counted using colony counter and recorded.

### Chemical Properties

### Determination of peroxide value

This was determined using the procedure described by **Okonkwo *et al*., (2014)** with slight modification. Briefly, 1 g of the sample was weighed into the boiling tube, followed by the addition of 1 g of potassium iodide and 20 mL of solvent mixture of acetic/chloroform mixture in 2:1 (v/v). The tube was placed in a boiling water bath such that the content was boiled within 30 s and continued boiling vigorously for more than 30 s. The reaction mix was quickly transferred into a flask containing 20 mL of 5 % KI solution, and the boiling tube rinsed twice with 25 mL of distilled water, each time. Using starch indicator, the system was titrated against 0.002 M Sodium thiosulphate solution. A blank titration was carried out, and peroxide value (mEq/kg) computed.

$$\text{Peroxide value} = \frac{\text{Blank}-\text{titre} \times 0.02\ \text{M} \times 1000}{\text{weight of sample}}$$

### Determination of free fatty acid

The procedure for the determination of (FFA) was carried out using the method described by **Chindo *et al*., (2010)** with slight modification. 2 g of the sample was weighed into a 250 mL conical flask and 10 mL of ethanol (95%) was added and the resulting mixture was titrated with 0.1 M sodium hydroxide using phenolphthalein as indicator. The titration was done with constant shaking until a pink color persisted for 30 seconds.

$$\%\ \text{FFA} = \frac{\text{V} \times \text{M} \times 2.82}{\text{weight of sample(g)}}$$

Where V (mL) = volume of sodium hydroxide solution used; M = molarity of sodium hydroxide solution used; 2.82 = conversion factor for oleic acid.

### Determination of protein

Themacro kjeldahl method as described by **AOAC (2005)** was used. About 0.2 g of the sample was accurately weighed into a conical flask (250 mL), 0.8 g of potassium sulphate was poured inside the conical flask. About 15 mL of sulphuric acid was poured inside and 3-4 glass bead (anti bumps) were dropped inside the conical flask and swirled. It was then heated on the kjeldhal apparatus for 2-3 h at approximately 100 $^{\circ}$C, until it turns bluish-white indicating freedom from any organic matter (end of protein digestion). The digest was allowed to cool in air and diluted with 10 mL distilled water. This was distilled using markham distillation apparatus where 100 mL conical flask containing 5 mL of boric and 2-3 drops of mixed indicator was attached and 5 mL of the digest was introduced into the body of the apparatus followed by 10 mL of 40- 45% sodium hydroxide solution and distillate collected as ammonium sulphate which was titrated against 0.1 M hydrochloric acid. A blank titration was carried out using distilled water instead of the distillate. Percentage nitrogen was calculated using the formula:

$$\text{Nitrogen} = \frac{\text{titre value}-\text{blank x } 0.0014 \text{ g N x } 100 \text{ x } 25}{\text{weight of sample x } 5 \text{ mL aliquot}}$$

Protein = % nitrogen × 6.25 (Conversion factor)

### Determination of TBA (Thiobarbituric acid test)

The procedure for the determination of TBA was carried out using the method described by **AOAC (2005)**. Sample was weighed into 25 mL volumetric flask and dissolved in small volume of 1- butanol. The mixture was made up to volume with 1- butanol followed by mixing. Then 5.0 mL of the mixture was pipetted into a dried stopper test tube, and then 5.0 mL of thiobabituric acid reagent was added followed by mixing. The mixture was placed in water bath at 95 ºC for 120 min, then cooled to room temperature and absorbance read at 450 nm in 10 mm cell.
The absorbance of the reagent blank as (AB) was also recorded. TBA is calculated as follows:

$$\text{TBA value (mg malonoldehyde / kg)} = \frac{50 \times (\text{AS}-\text{AB})}{\text{M}}$$

M = weight of sample
AS = Absorbance reading of sample solution
AB = Absorbance reading of blank solution

### Sensory Evaluation

Sensory evaluation based on appearance, odour, texture and general acceptability was done by ten trained panelist been students from the department of Food Science and Technology, University of Agriculture, Makurdi using the rating scale shown below.

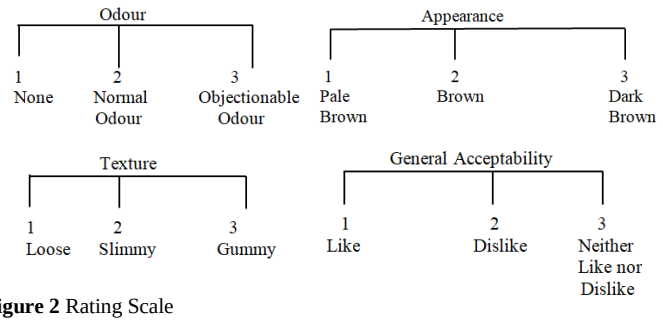


**Figure 2** Rating Scale

**Statistical Analysis**

The data obtained were subjected to Analysis of Variance (ANOVA) and Duncan Multiple range test was used to separate means where
Significant differences existed and data analyses were achieved using the Statistical Package for Social Statistics (SPSS) software version 20.0.

**RESULTS AND DISCUSSION**

**pH and microbial count of the samples**

The pH values ranging from 6.68 to 9.23 increased from the acid state into the alkaline state with increase in *Bacillus subtilis* concentration and fermentation time as presented in Table 2. Similar trend were also reported by **Omafuvbe (2006)** and **Adelekan and Nwadiuto (2012).** There was significant difference ($p< 0.05$) in the pH of the samples except for samples, C and D. The observed increase in pH during fermentation time could be attributed to the ability of *Bacillus subtilis* to hydrolyze protein into amino acid and ammonia. An increase in the ammonia been liberated led to a corresponding increase in the pH. The level of increase follows the same trend as obtained by **Adelekan and Nwadiuto (2012) and Odunfa (1985)** for locust bean fermentation. The total viable counts of the fermented locust bean were insignificant to be counted at 24 h fermentation period. This may be due to the fact that the *Bacillus subtilis* inoculated were just getting adapted to their new environment. As the pH increased with increase in fermentation time, *Bacillus subtilis* got more adapted to their environment and multiplied at their maximum rate with 72 h having the highest bacterial count. Thus, the longer the fermentation time the higher the bacterial load.

**Table 2** pH values and total viable count of the fermented locust bean samples

| **Samples** | ***B. subtilis* concentration (in mL)** | **pH** | **TVC (cfu/g)** |
|---|---|---|---|
| Rawseed | | $6.00^{a}\pm0.01$ | ____ |
| 24 h fermentation | 1.0 | $6.68^{b}\pm0.03$ | <30 |
| | 1.5 | $6.94^{c}\pm0.04$ | <30 |
| 48 h fermentation | 1.0 | $8.87^{d}\pm0.03$ | $2.5x10^{5}$ |
| | 1.5 | $8.91^{d}\pm0.02$ | $3.3x10^{5}$ |
| 72 h fermentation | 1.0 | $8.96^{e}\pm0.01$ | $2.9x10^{5}$ |
| | 1.5 | $9.23^{f}\pm0.02$ | $3.7x10^{5}$ |

Values for pH are means ± standard deviation of three replicates. Mean with different letters within columns are significantly different ($p<0.05$).
**Legend:** A = 24 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, B= 24 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, C= 48 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, D= 48 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, E= 72 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, F= 72 h fermented locust beans at 1.5 mL concentration of *B. subtilis* and cfu/g= coliform forming units per gram. TVC= Total Viable Count

**Effect of *Bacillus subtilis* concentration and fermentation time on the chemical properties of locust beans condiment**

**Peroxide value**: There were significant differences ($p< 0.05$) in the peroxide values of all the entire samples (Table 3). The peroxide value increased with increase in fermentation time and *Bacillus subtilis* concentration with 72 h having the highest peroxide value. Peroxide value can be regarded as a biomarker or an index of lipid oxidation. The most susceptible to oxidation are the poly unsaturated lipid; because of the presence of double bonds which makes the fatty acid molecules reactive with formation of free radicals and derive oxidize compounds **(Omafuvbe *et al*., 2004)**. The relatively higher content of poly unsaturated fatty acid in the fermented locust beans at 72 h may account for higher lipid oxidation that could lead to high rancidity and off flavor.
**FFA**: The Free fatty acid (FFA) of locust beans condiment increased progressively with fermentation time as shown in Table 3. There was significant difference in the FFA values of the sample. However, the free fatty acid at 72 h fermentation time was significantly higher indicating a higher breakdown of fat molecules during fermentation. This suggests that rancidity and off flavor may have set in earlier and faster at 72 h fermentation. The increased trend observed in this study was similar to those reported by **Kolapo *et al*., (2007)**. This also collaborates the decision of the panelist in out rightly rejecting the condiments fermented for 72 h due to the offensive odour associated with the food product at this stage.

**Protein:** The protein content of the fermented condiment decreased from 28.14 to 22.01% as fermentation time and *Bacillus subtilis* concentration increased (Table 3). There was significance difference ($p<0.05$) in the protein values of all the samples. The decline in the protein content could be attributable to the high proteolytic activities of *Bacillus subtilis*. *Bacillus subtilis* is known to break down more of protein into amino acids and ammonia as fermentation increases **(Omafuvbe *et al*., 2002)**. Condiment fermented for 72 h had the lowest protein value of 22.01 which implies that more of the amino acids had been degraded into ammonia. This finding disagrees with the report of **Omafuvbe *et al*., ( 2004)** who reported an increase in protein during fermentation. The author however, claimed the increase may probably be due to the reduction in the content of ash, crude fiber and carbohydrate. An excessive degradation of protein into ammonia is known to be responsible for the offensive smell normally associated with fermented locust beans condiments. This should be of great concern to the food processor. In order to produce a very minimal or ammonia-free locust bean condiment, there is need to ensure an effective balance of the *Bacillus subtilis* concentration and fermentation time. One key benefit of using B. *subtilis* as a sole fermenting agent during locust bean fermentation is its ability to act as a probiotic organism in food. When conditions are favourable, it exhibits its antibiotic potentials and when conditions become unfavourable, it could form spores.

**TBA:** TBA value/number estimates the amount of malanoldehyde (MDA) present in a lipid sample **(Okonkwo *et al*., 2014)**. The TBA of the samples increased from 1.40 to 1.95 mg malanoldehyde/ kg as the fermentation time and *Bacillus subtilis* concentration increased. There was significance difference in all the entire samples with samples, E and F having the highest TBA values. The relatively higher content of TBA value may be reasonable for the higher lipid oxidation leading to rancidity and off flavour of the condiment.

**Table 3** Effect of *Bacillus subtilis* concentration and fermentation time on the physico-chemical properties of fermented locust beans condiment

| **Samples** | **Peroxide Value (mEq/kg)** | **FFA (%)** | **Protein (%)** | **TBA(mg malanoldehyde/ kg)** |
|---|---|---|---|---|
| A | $0.40^{a}\pm0.01$ | $0.13^{a}\pm0.01$ | $28.14^{f}\pm0.02$ | $1.40^{a}\pm0.01$ |
| B | $0.43^{b}\pm0.01$ | $0.15^{b}\pm0.00$ | $27.84^{e}\pm0.01$ | $1.43^{b}\pm0.01$ |
| C | $1.67^{c}\pm0.03$ | $0.24^{c}\pm0.00$ | $24.80^{d}\pm0.01$ | $1.44^{c}\pm0.02$ |
| D | $1.74^{d}\pm0.01$ | $0.26^{d}\pm0.00$ | $24.10^{c}\pm0.01$ | $1.47^{d}\pm0.01$ |
| E | $4.60^{e}\pm0.04$ | $0.29^{e}\pm0.01$ | $22.65^{b}\pm0.02$ | $1.92^{e}\pm0.01$ |
| F | $5.00^{f}\pm0.02$ | $0.32^{f}\pm0.00$ | $22.01^{a}\pm0.01$ | $1.95^{f}\pm0.01$ |

Values are means ± standard deviation of three replicates. Mean with different letters within columns are significantly different ($p<0.05$).
**Legend:** A = 24 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, B= 24 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, C= 48 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, D= 48 h fermented

locust beans at 1.5 mL concentration of *B. subtilis*, E= 72 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, F= 72 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, FFA= Free fatty acids and TBA= Thiobarbituric acid

**Effect of *Bacillus subtilis* concentration and fermentation time on the sensory attributes of fermented locust beans condiment**

The sensory scores for the fermented locust bean condiment are shown in Table 4. For appearance of the fermented condiment, panelist saw no significant difference in the condiment inoculated with 1.0 mL concentration of *B. subtilis* and fermented for 48 h and 72 h respectively. However, a significant difference was shown to exist in the other fermented condiment samples. A condiment of an acceptable quality should neither be dark nor completely brown, but dull brown with tinted white patches which may suggest the secretion of metabolites by the fermenting organisms. The interaction of amino acids and sugars during fermentation is said to be responsible for color formation **(Omafuvbe *et al*., 2004)**. Amino acids are also known to be responsible for the flavor of the condiment. However, excessive fermentation results in the degradation of amino acids into ammonia giving rise to the darkening of the condiment **(Achi, 2005)**. The condiment appearance continued to change progressively as the fermentation time change. The increased activities of the fermenting organism (*B. subtilis*) may be responsible for the change observed in this study. Odour is one of the key quality indices of the fermented condiments that decide their sensory acceptability to the consumers. Most consumers prefer condiments that have no offensive odour before or even after taste. From Table 4, a significant difference ($p<0.05$) exist between the odour of condiment treated and fermented for 72 h and those fermented for 24 h and 48 h respectively. Concentration of *B. subtilis* did not have effect on the odour of the products. However, prolonged fermentation to 72 h significantly affected the odour of the product. Panelist therefore, rated the products fermented for 72 h to be inferior in odour with unpleasant smell. The texture of locust bean condiment changed with the level of fermentation. Before the start of fermentation, the cotyledon is normally loose. As the fermentation progresses, with the secretion of metabolites, the product becomes ropy and slimy with strands of filament-like threads crisscrossing the bean cotyledons, the filament collapses creating a gummy substances that adheres to the bean cotyledon making them to stick together. From the result as shown in Table 4, the concentration of *B. subtilis* had no significant effect on the texture of the locust bean condiment. However, the fermentation time had a significant influence on the texture of the condiment. Condiment fermented for 48 h to 72 h significantly differs in their texture.

**Table 4** Sensory scores for *B. subtilis* fermented locust bean condiment

| Samples | Appearance | Odour | Texture | G. Acceptability |
|---|---|---|---|---|
| A | $1.4^{c}\pm0.16$ | $1.9^{b}\pm0.10$ | $1.7^{c}\pm0.15$ | $2.0^{a}\pm0.18$ |
| B | $1.6^{bc}\pm0.21$ | $2.0^{b}\pm0.00$ | $1.9^{bc}\pm0.17$ | $1.7^{bc}\pm0.15$ |
| C | $2.0^{ab}\pm0.00$ | $2.0^{b}\pm0.00$ | $2.5^{ab}\pm0.17$ | $1.2^{b}\pm0.13$ |
| D | $2.0^{b}\pm0.00$ | $2.0^{b}\pm0.15$ | $2.6^{a}\pm0.16$ | $1.8^{ab}\pm0.30$ |
| E | $2.0^{ab}\pm0.00$ | $2.8^{a}\pm0.13$ | $3.0^{a}\pm0.10$ | $2.3^{a}\pm0.22$ |
| F | $2.1^{a}\pm0.10$ | $2.8^{a}\pm0.13$ | $3.0^{a}\pm0.10$ | $2.3^{a}\pm0.22$ |

Values are means ± standard deviation of three replicates. Mean with different letters within columns are significantly different ($p<0.05$).

**Legend:** A = 24 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, B= 24 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, C= 48 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, D= 48 h fermented locust beans at 1.5 mL concentration of *B. subtilis*, E= 72 h fermented locust beans at 1.0 mL concentration of *B. subtilis*, F= 72 h fermented locust beans at 1.5 mL concentration of *B. subtilis*

**CONCLUSION**

The use of *Bacillus subtilis* for fermentation of locust bean was found to be concentration and time dependent. The higher the concentration of *Bacillus subtilis* and fermentation time the more the fermentation activities. Based on the aforementioned results, it could be concluded that excessive fermentation activities of *Bacillus subtilis* were undesirable at 72 h as this gave rise to low quality locust bean condiment with unpleasant odour. This research showed that *Bacillus subtilis* concentration of 1 mL and a fermentation period of 48 h produced a condiment with higher degree of consumer acceptability with no unpleasant odour. The next research approach on this product should aim at arresting the activities of *Bacillus subtilis* at an appropriate time to make them dormant while still serving as probiotics.

**Conflicts of interest:** All authors have no conflicts of interest to declare.

**REFERENCES**


ACHI, O. 2005. Traditional fermented protein condiments in Nigeria. *African Journal of Biotechnology, 4*(13), 1612-1621

ACHINEWHU, S. 1983a. Chemical and nutrient composition of fermented products from plant foods. *Nig. Fd. J.,, 1*, 115-116.

ACHINEWHU, S. 1983b. Protein Quality of African Oil Bean Seed (*Pentaclethra machrophylla*). *Journal of food science, 48*(4), 1374-1375. https://dx.doi.org/10.1111/j.1365-2621.1983.tb09241.x

ADEGOKE, G. 2004. *Understanding food Microbiology* (2 ed.). Nigeria: Alleluia Venture Ltd.

ADELEKAN, A. O., NWADIUTO, E. 2012. Bacterial succession studies during fermentation of African locust bean (*Parkia biglobosa*) to iru using molecular methods. *British Biotechnology Journal, 2*(1), 49-59. https://dx.doi.org/10.9734/bbj/2012/586

AJAYI, O. A. 2014. Bacteriology and qualitative study of African locust bean (*Parkia biglobosa*). *Open Journal of Social Sciences, 2*(11), 73. https://dx.doi.org/10.4236/jss.2014.211010

AKINRELE, I. 1970. Fermentation studies on maize during the preparation of a traditional African starch-cake food. *Journal of the Science of Food and Agriculture, 21*(12), 619-625. https://dx.doi.org/10.1002/jsfa.2740211205

AOAC. (2005). *Official Methods of Analysis, Association of Official Analytical Chemist* (19 ed.). Washington DC.

CAMPBELL-PLATT, G. 1980. African locust bean (Parkia species) and its West African fermented food product, dawadawa. *Ecology of food and nutrition, 9*(2), 123-132. https://dx.doi.org/10.1080/03670244.1980.9990590

CHELULE, P., MOKOENA, M., GQALENI, N. 2010. Advantages of traditional lactic acid bacteria fermentation of food in Africa. *Current Research, Technology and Education Topics in Applied Microbiology and Microbial Biotechnology, 2*, 1160-1167.

CHINDO, I., GUSHIT, J., OLOTU, P., MUGANA, J., TAKBAL, D. 2010. Comparism of the quality parameters of the seed and condiment oil of *Adansonia digitata*. *Journal of American Science, 6*(12), 990-994.

DUNSIN, O., AJIBOYE, G., ADEYEMO, T. 2014. Effect of alternative hormones on the rootability of *Parkia biglobosa. Journal of Agriculture, Forestry and the Social Sciences, 12*(2), 69-77. https://dx.doi.org/10.4314/joafss.v12i2.8

EKUNDAYO, J. 1977. *Nigerian pito*. Paper presented at the Symposium and Indigenous Fermented Foods, Bangkok, Thailand.

GADAGA, T. H., SAMENDE, B. K., MUSUNA, C., CHIBANDA, D. 2008. The microbiological quality of informally vended foods in Harare, Zimbabwe. *Food control, 19*(8), 829-832. https://dx.doi.org/10.1016/j.foodcont.2007.07.016

GBERIKON, G., AGBULU, C. 2015. Benefits of Utilizing Starter Cultures in the Fermentation of *Glycine max* for Production of Condiment in the Food Industry. *Research Journal of Microbiology, 10*(1), 33-37. https://dx.doi.org/10.3923/jm.2015.33.37

IFEANYIEZE, F., NWAPKADOLU, G., NWAREJI, F. 2016. Business benefit concerns of women processors of African locust been seeds for poverty reduction in Enugu State-Nigeria. *Global Journal of Agricultural Research 4*(4), 47-56.

IWUOHA, C. I., EKE, O. S. 1996. Nigerian indigenous fermented foods: their traditional process operation, inherent problems, improvements and current status. *Food Research International, 29*(5-6), 527-540. **https://dx.doi.org/10.1016/0963-9969(95)00045-3**

KOLAPO, A., POPOOLA, T., SANNI, M., AFOLABI, R. 2007. Preservation of soybean *daddawa* condiment with dichloromethane extract of ginger. *Research Journal of Microbiology, 2*(3), 254-259. https://dx.doi.org/10.3923/jm.2007.254.259

KUBOYE, A. 1985. *Traditional fermented foods and beverages of Nigeria*. Paper presented at the Proceedings of the IFA/UNU workshop on the development of indigenous fermented foods and food technology in Africa, Douala, Cameroon. Stockholm: International Foundation for Science.

LARANJO, M., ELIAS, M., FRAQUEZA, M. J. 2017. The use of starter cultures in traditional meat products. *Journal of Food Quality, 2017*. https://dx.doi.org/10.1155/2017/9546026

MARCO, M. L., HEENEY, D., BINDA, S., CIFELLI, C. J., COTTER, P. D., FOLIGNÉ, B., GÄNZLE, M., KORT, R., PASIN, G., PIHLANTO, A., SMID,

E.J., HUTKINS, R. 2017. Health benefits of fermented foods: microbiota and beyond. *Current Opinion in Biotechnology, 44*, 94-102. https://dx.doi.org/10.1016/j.copbio.2016.11.010

MOHAMMED, S., ORUKOTAN, A., & MUSA, J. 2017. Effect of fermentation and malting on some cereal weaning foods enriched with African locust beans. *Journal of Applied Sciences and Environmental Management, 21*(5), 911-921. https://dx.doi.org/10.4314/jasem.v21i5.17

ODEBUNMI, E., OLUWANIYI, O., BASHIRU, M. 2010. Comparative proximate analysis of some food condiments. *Journal of Applied Sciences Research, 6*(3), 272-274.

ODUNFA, S. 1985. Biochemical changes in fermenting African locust bean (*Parkia biglobosa*) during '*iru*'fermentation. *International Journal of Food Science & Technology, 20*(3), 295-303. https://dx.doi.org/10.1111/j.1365-2621.1985.tb00379.x

ODUNFA, S., OYEYIOLA, G. 1985. Microbiological study of the fermentation of ugba, a Nigerian indigenous fermented food flavour. *Journal of Plant foods, 6*(3), 155-163. https://dx.doi.org/10.1080/0142968x.1985.11904309

OGUNDIWIN, J. 1978a. *A study of milk clotting by the vegetable renin from the leaves of Sodom apple (Culotropis procera).* . Paper presented at the In Proc. of the 2nd Ann. Conf.' of NIFST, Lagos, 7-9 September, 1978, Lagos.

OJINNAKA, M., OJIMELUKWE, P., EZEAMA, C. 2013. Changes in enzyme activities during the fermentation of castor oil bean seeds using B. subtilis as monoculture starter. *African Journal of Food Science and Technology, 4*(5), 122-128.

OKONKWO, T. J., OBONGA, W. O., OKONKWO, C. J. 2014. Fatty Acid Profile and Physicochemical Properties of *Landolphia owariensis* P. Beauv Stringy Seed Oil. *Tropical Journal of Pharmaceutical Research, 13*(8), 1347-1352. https://dx.doi.org/10.1055/s-0033-1352167

OKORIE, C. P., OLASUPO, N. A. 2013. Controlled fermentation and preservation of *UGBA*–an indigenous Nigerian fermented food. *SpringerPlus, 2*(1), 470. https://dx.doi.org/10.1186/2193-1801-2-470

OLASUPO, N., SMITH, S., AKINSINDE, K. 2002. Examination of the microbial status of selected indigenous fermented foods in Nigeria. *Journal of Food Safety, 22*(2), 85-93. https://dx.doi.org/10.1111/j.1745-4565.2002.tb00332.x

OMAFUVBE, B. 2006. Effect of salt on the fermentation of soybean (*Glycine max*) into daddawa using *Bacillus subtilis* as starter culture. *African Journal of Biotechnology, 5*(10), 1001-1005.

OMAFUVBE, B., ABIOSE, S., SHONUKAN, O. 2002. Fermentation of soybean (*Glycine max*) for soy-daddawa production by starter cultures of Bacillus. *Food Microbiology, 19*(6), 561-566. https://dx.doi.org/10.1006/fmic.2002.0513

OMAFUVBE, B. O., FALADE, O. S., OSUNTOGUN, B. A., ADEWUSI, S. R. 2004. Chemical and biochemical changes in African locust bean (*Parkia biglobosa*) and melon (*Citrullus vulgaris*) seeds during fermentation to condiments. *Pakistan Journal of Nutrition, 3*(3), 140-145. https://dx.doi.org/10.3923/pjn.2004.140.145

OMODARA, T., OLOWOMOFE, T. 2015. Effects of fermentation on the nutritional quality of African locust bean and soybean. *Int. J. Sci. Res, 4*, 1069-1071.

ONYEKWERE, O. 1977. *The processing of roots and tubers.* Paper presented at the In Proc. of 1st Ann. Conf of NIFST, Lagos, 5-7 May, 1977.

PIERSON, M., REDDY, N., ODUNFA, S. 1986. *Legume-based Fermented Foods*. Boca Raton, Florida: CRC Press.

SIMONYAN, K. J. 2012. *African Locust bean seed processing* (1st ed.). Lagos: Naphtali Prints Nig. Ltd.

TEE, T., OGWUCHE, J., IKYAAGBA, E. 2009. The role of locust bean and ironwood trees in human nutrition and income in Nigeria. *Pakistan Journal of Nutrition, 8*, 1172-1177. https://dx.doi.org/10.3923/pjn.2009.1172.1177

TEHINSE, I., OGUNDIWIN, J. 1978. A study of some quality characteristics of *Otika*–A traditional alcoholic beverage from guinea corn. *Nigerian Institute of Food Science and Technology, 2*, 61-70.

UZOGARA, S., AGU, L., UZOGARA, E. 1990. A review of traditional fermented foods, condiments and beverages in Nigeria: Their benefits and possible problems. *Ecology of food and nutrition, 24*(4), 267-288. https://dx.doi.org/10.1080/03670244.1990.9991145