

COMPARISON OF THE ANTIOXIDANT AND PHYTOCHEMICAL PROPERTIES OF DAWADAWA (SUB-SAHARA AFRICA CONDIMENT) FROM *MUCUNA PRURIENS* L. AND *PARKIA BIGLOBOSA* Jacq. UNDER CONTROL CONDITION

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ABSTRACT

The ethnopharmacological relevance of *Mucuna pruriens* (Linn) with its' nutrients content present itself as an option in functional food. This study evaluated the antioxidant and phytochemical properties of *Bacillus subtilis* fermented condiment (dawadawa) from *Mucuna pruriens* in comparison with *Parkia biglobosa* using dehulled, thermally treated seeds. The phytochemicals decreased with increase in pressured cooking time; with the condiment of *Parkia* having higher significant difference. The total phenol, vitamin C and total flavonoid (TF) content for *Mucuna* (123.34 mg/g; vit C-0.72 ±0.04 mg/g; TF-22.21 ±0.23 mg/g) were significantly higher than *Parkia*'s. The FRAP and ABTS scavenging ability of *Parkia* decreased with cooking time, while that of *Mucuna* (34.33 ±1.49 mg/g and 98.33 ±1.39 millimol/g) was increasing. Fermented *Mucuna* (50.73 %) demonstrated higher ability to scavenge 1, 1- diphenyl-2-picrylhydrazyl radicals. Result obtained suggested that fermented *Mucuna* may produce valuable contribution to health as an antioxidant in comparison to fermented *Parkia* as nutraceutical.

Keywords: antioxidant, condiment, nutraceutical, *Mucuna pruriens*, phytochemical

INTRODUCTION

Many of the known wild and underutilized legumes (such as *Mucuna* spp., *Canavalia* spp., *Sesbania* spp., etc.) possess adequate amounts of protein, essential amino acids, polyunsaturated fatty acids (PUFAs), dietary fiber, essential minerals and vitamins along with the presence of beneficial bioactive compounds when comparable to other common legumes (Bhat and Karim, 2009). *Mucuna pruriens* (*M. pruriens*), a leguminous plant, is identified as an herbal medicine for improving the fertility and related disorders in the Indian traditional system of medicine, however without any scientific validations. All part of *Mucuna* plants are known to possess high medicinal value. The seeds have been known to contain many pharmaceutical with antiepileptic and anti-neoplastic activities. It also possesses anti-parkinsonian activities, anti-inflammatory, antispasmodic, anti-vemine, anabolic, androgenic, analgesic, aphrodisiac, (Guerranti et al., 2002). Raw *Mucuna* bean seed is known to contain anti-nutritional/toxic factors such as trypsin inhibitors, tannins and cyanide. The presence of Dopa (a potentially neurotoxic agent) in the raw bean has also been reported (Siddhuraju et al., 1996). The plant is rich in alkaloids such as prurienine, prurienine and prurienidine (Misra and Wagner, 2007). Triterpenes and sterols (-sitosterol, ursolic acid, etc.) are found in the root and seeds of *M. pruriens*. The seeds also contain amino acids methionine, tyrosine, lysine, glycine, aspartic acid, glutamic acid, leucine and serine along with globulins and albumins, fatty acids, carbohydrates, and related compounds such as oleic acid, linoleic acid and palmitic acid (Adebowale et al., 2005).

The anti-nutritional toxic factor has to be decreased to safe level either by boiling, heating or fermentation before the inclusion of any of the unconventional legumes in livestock rations (Josephine and Janardhanan, 1992). Another method of improving nutritional quality of legumes is fermentation. Food fermentation is a microbial and enzymatic method for food processing to achieve prolonged shelf-life. Oil seeds such as African locust bean, melon seed, castor oil seed, mesquite seed and soybean are also fermented to produce condiments. African locust bean tree *Parkia biglobosa* is widely recognized in West Africa as an important multipurpose tree of West Africa Savannah land. The most important part of the tree is found in its seeds and the processed seed is used as condiment for soup. It is a source of natural nutritious condiment which features frequently in the traditional diet of the people (Fagbemi, 1989). Food condiments or spices are strong-smelling, sharp tasting substances usually used to improve or adjust the flavour of food. They are usually of vegetable origin which common examples

include mustard, nutmeg, ginger, garlic, coriander, locust bean. Alkaline fermentations cause the hydrolysis of protein to amino acids and peptides and releasing ammonia, which increases the alkalinity by the *Bacillus* species such as *Bacillus subtilis* (dominant species), *B. licheniformis* and *B. pumilus* (Enujiugha et al., 2008; Chelule et al., 2010). *Bacillus subtilis* is a strongly proteolytic bacterium which is mainly used in alkaline fermentation of legumes such as soybean. It causes biochemical changes in beans by hydrolysis of proteins and metabolism of resultant amino acids leading to increase in pH and flavour development. In order to improve nutritional quality, and effective utilization of dry legumes to their full potential as food, inactivation or removal of anti-nutritional factors by adopting economically viable processing techniques is necessary. *Mucuna pruriens* been reported as a high proteineous crop than most commonly consumed legumes content with a ranges of 23 to 35%. Therefore this study is undertaken to investigate the possibility of using *Mucuna pruriens* as an alternative to *Parkia biglobosa* seed for the production of condiments. Also to assess the quality of condiments produced from the two beans using starter culture of *Bacillus subtilis* 7A and BCD 333.

MATERIALS AND METHODS

Sample collection

The velvet beans (*Mucuna pruriens*) and locust beans (*Parkia biglobosa*) used for this study were purchased from a research institute (IITA) in Ibadan, Oyo State and open market in Akure, Ondo State, Nigeria respectively. All reagents used for the analysis were of analytical grade.

Preparation of samples

Harvest of microbial cells

Two strains of *Bacillus subtilis* (7A and BCD 333) selected based on favourable morphological, biochemical characteristics and dominance after fermentation were maintained on broths and slants (Aderibigbe et al., 2011). Fresh cell cultures of *Bacillus subtilis* (7A and BCD 333) isolates which were activated overnight in nutrient agar respectively were harvested in graduated sterilized Eppendorf tubes of 5ml using a micro centrifuge (Stuart microfuge SRFC1 10000 X rpm) at

6000rpm for 5min. After centrifuging the supernatants were decanted using micropipette and sterilized tips. The cell pellets at the bottom of the tubes were rinsed with 1000 µl sterile DNA free water, centrifugation was repeated at 6000 rpm for 3min. After which the supernatants were decanted and the cell pellets were made up with 1000µl of sterile DNA free water to obtain pure strains cells. The harvested cells were maintained on ice prior to usage.

Production of fermented beans from *Parkia biglobosa* and *Mucuna pruriens*

The modified method of Gabriel *et al.*, (2004) was employed. The locust and velvet beans seeds were sorted to remove dirt, stones and foreign materials. The locust beans were cooked for 2 h after which they were dehulled using mortar and pestle and washed in clean water until all the hulls have been removed. Alternatively, the velvet seeds were soaked in hot water for ten min and thereafter, dehulled manually. The dehulled seeds of each beans were then divided into three portions; the first portion was not pressure cooked after dehulling, while the remaining two portions were pressure cooked for 15 min and 30 min respectively. The uncooked dehulled sample was soaked in 10 % hypochlorite to make it sterile after which, it was aseptically transferred into a sterile foil plate and the inoculum was added to it under aseptic conditions. The remaining two pressured-cooked portions were also transferred aseptically into sterile foil plate after which, the inoculums (*Bacillus subtilis* 7A and BCD 333) used were added respectively and these were kept in plastic buckets and fermented at 35 °C inside an incubator respectively for 36 hr. The resulting fermented seeds were dried in an oven (PEC North America, Boca Raton, FL 33431, USA) at 60 °C for 6 h. It was then milled and stored for analysis.

Determination of total viable microbial count of fermenting beans

Ten grams of fermented seeds was homogenized with 0.1 % sterile peptone water (Biolab Zrt., Hungary) in sterile Ziploc bag working under aseptic condition and serial dilution was performed. One milliliter of diluents five and six were aseptically dispensed into sterile Petri dish with sterilized Nutrient agar (Lab M, Lancashire BL9 7JJ, United Kingdom) added and swirled for proper mixing. It was allowed to solidified and incubated at 37 °C for 18 h. The total viable count during the fermentation period was determined with the help of a colony counter (Deihan Labtech, New Delhi-110008, India) and the colony forming unit (cfu) calculated. This was done at every 12 h interval throughout the 36 h of fermentation time.

Physicochemical analyses of fermented velvet and locust beans

The pH of the samples was determined at every 12 h interval throughout the fermentation period of 36 h. Two gram of homogenized fermenting beans was taken using a pH meter (Model 401).

The amount of lactic acid in the fermenting mass was determined by titration of 20 ml filtrate obtained from 2 g of fermenting seeds dissolved in 20 ml distilled water against 0.1 M NaOH using phenolphthalein as indicator. The titre value was then used to calculate the titratable acidity as percentage lactic acid (AOAC, 2010).

Determination of phytochemical composition of fermented velvet and locust beans

Oxalate was determined according to the titration method of Day and Underwood, (1986). 1g of sample was weighed into 100ml conical flask. 75ml 3M₂SO₄ was added and stirred for 1hr with a magnetic stirrer. 25ml of the filtrate was then taken and titrated while hot against 0.05M KMnO₄ solution until a faint pink colour persisted for at least 30 sec. The oxalate content was then calculated by taking 1ml of 0.05m KMnO₄ as equivalent to 2.2mg oxalate.

The spectrophotometric method of Brunner, (1984) was used for saponin quantification. Two gram of the finely grinded sample was weighed into a 250 mL beaker and 100 mL of Isobutyl alcohol was added, the mixture was shaken in the Shaker for 5 h to ensure uniform mixing. The mixture was filtered into 100 mL beaker containing 20 mL of 40% saturated solution of magnesium carbonate. The mixture obtained again was filtered to obtain a clean colourless solution. One milliliter was added into 50 mL volumetric flask using pipette, 2 mL of 5% iron (iii) chloride (FeCl₃) solution was added and made up to the mark with distill water. It was allowed to stand for 30 min for the colour to develop. The absorbance was read against the blank at 380 nm:

Phytate was determined according to the method of Okon and Akpanyung (2005). 100ml of the sample was extracted with 3% trichloroacetic acid. The extract was treated with FeCl₃ solution and the iron content of the precipitate was determined using Atomic Absorption spectrophotometer (Cye Unicam 2900). A 4:6 Fe/P atomic ratio was used to calculate the phytic acid content.

Trypsin inhibitor activity (TIA) was determined by the method of Kakade *et al.*, (1974) using benzoyl-DL-arginine-*p*-nitroanilide (BAPNA) as substrate. Absorbance (A) was read at 410 nm wavelength. Trypsin inhibitory activity was defined as the number of trypsin inhibitor units (TIU). One TIU was defined as an increase of 0.01 in absorbance units under conditions of assay.

Tannin was determined according to the method of Makkar (1999). Exactly 2.0 g of sample was added to 20 mL of 50% methanol and placed in a water bath at 77°C

- 80°C for 1 hr and shaken. The extract was quantitatively filtered using a double layered Whatman No.1 filter paper and 20 mL of distilled water, 2.5 mL Folin-Denis reagent and 10 mL 17% Na₂CO₃ were added and mixed. The mixture was allowed to stand for 20 min. The bluish-green colour developed at the end of the treatment on the range 0 - 10 ppm. The absorbance of the tannic acid standard solutions as well as samples was read after colour development on a spectronic 21D spectrophotometer at a wavelength of 760 nm against a reagent blank concentration of the same solution from a standard tannic acid curve prepared. Percentage tannin was calculated.

Determination of antioxidant properties of fermented velvet and locust beans

For evaluation of antioxidant activity of the fermented flours, all tests were carried out in triplicate. The extraction technique of Oboh *et al.*, (2007) was used to collect supernatant from 1.0 g of each fermented sample using the rotary shaker (Centurium Scientific, United Kingdom) at 190 rpm-220 rpm for 3 h, filtered, and centrifuged (Centurium Scientific, United Kingdom) at 5000 rpm for 15 min.

This was used in antioxidant measurements and determination of total phenolic (Singleton *et al.*, 1999). The reaction mixture composed of 0.5 ml of methanolic solution of extract, 2.5 ml of 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml 7.5% NaHCO₃. The blank contained 0.5 ml methanol, 2.5 ml 10% Folin-Ciocalteu's reagent dissolved in water and 2.5 ml of 7.5% of NaHCO₃. The samples were thereafter incubated in a thermostat at 45 oC for 45 min and absorbance taken at 765 nm using spectrophotometer. The samples were prepared in triplicate for each analysis and the mean value of absorbance was obtained. Gallic acid was used as the standard solution and calibration line was construed. Based on the measured absorbance, the concentration of phenolics was read (mg/ml) from the calibration line; then the content of phenolics in extracts was expressed in terms of gallic acid equivalent (mg of GA/g of extract).

The flavonoid contents (Bushra *et al.*, 2009) modified by incubating at room temperature for 30 min. Extracts were diluted with water in a 10 ml volumetric flask. Initially, 5% NaNO₂ solution was added to each volumetric flask; at 5 min, 10% AlCl₃ was added; and at 6 min, 1.0 M NaOH was added. Water was then added to the reaction flask and mixed well. Absorbance of the reaction mixture was read at 430 nm. The results were expressed in mg quercetin/g DW by comparison with the quercetin standard curve, which was made in the same condition.

Ferric reducing antioxidant power (FRAP) assay was determined according to the method of Oyaizu, (1986) and the absorbance read at 700 nm in UV-Visible spectrophotometer (PG Instruments, Leicestershire LE17 5BH, United Kingdom). The sample extracts of varying concentration (20–100 µg) were taken in 1 mL of phosphate buffer in a test tube and 5 mL of 0.2 M phosphate buffer, pH 6.6 was added. To this, 5 mL of 1% potassium ferricyanide solution was added. The mixture was incubated at 50 °C for 20 min. After the incubation, 5 mL of 10% TCA was added and the content was centrifuged at 1,000 rpm for 10 min. The upper layer of the supernatant (5 mL) was mixed with 5 mL of distilled water. To this, 1 mL of ferric chloride (0.1%) was added and vortexed. Then, the absorbance of the reaction mixture was read spectrophotometrically at 700 nm against water blank.

The spectrometric assay uses the stable radical 2, 2-diphenyl-1-picryl-hydrazyl (DPPH) as a reagent (Butrits and Bucar, 2000) by the hydrogen atom or electrons donating ability of the fermented beans sample. Alcohol solution of DPPH (3 ml) was added to 1 ml samples containing different concentrations originating from the extracts. The samples were first kept in a dark place at room temperature and their absorbance was read at 518 nm after 30 min. The antiradical activity (AA) was determined using the below formula: AA% = ((Absorbance sample - Absorbance empty sample) × 100) / Absorbance control. Blank samples contained 1 ml ethanol + 2.5 ml from various concentrations of plant extract. Control sample containing 1 ml of 0.3 mM DPPH + 2.5 ml ethanol. The optic density of the samples, the control and the empty samples were measured in comparison with ethanol. BHT (butylhydroxytoluene) and α-tocopherol were used as positive controls.

Ascorbic acid (Vitamin C) content was analyzed by the modified spectrophotometric method described by Güçlü *et al.*, (2005). The method used is based on the oxidation of Ascorbic acid to dehydroascorbic acid with a Cu(II)-Nc reagent in an ammonium acetate-containing medium at pH 7.0, where the maximum absorption wavelength of the formed bis-(neocuproine) copper(I) chelate is 450 nm. One mL of CuCl₂ solution was placed in a test tube, and then 1 mL of Nc, and 1 mL ammonium acetate to bring the final pH to 7.0; 1.4 mL of water and 0.6 mL Ascorbic acid solution were finally added by mixing in this order. After 2 min, the absorbance at 450 nm was recorded against a reagent blank. The 2, 2'-Azino-bis (3-ethylbenzothiazoline-6-sulfonic) acid (ABTS) antioxidant activity was assayed on the basis of a protocol represented by Re *et al.*, (1999) spectrophotometrically at a wave length of 734 nm. The percentage of the radical scavenging capacity was determined. ABTS was dissolved in water to a 7 mM concentration. ABTS radical cation was produced by reacting ABTS stock solution with 2.45 mM potassium persulfate (final concentration) and allowing the mixture to stand in the dark at room temperature for 12–16 h before use. Prior to assay, the solution was diluted in ethanol (about 1:89 v/v) and equilibrated to 30 °C to give an absorbance at 734 nm of 0.700±0.02 in a 1 cm cuvette. The concentration of sample extract that produced between 20% and 80% inhibition of the blank

absorbance was determined and adapted. Triplicate determinations were made at each dilution of the standard, and the percentage inhibition of the blank absorbance at 734 nm was plotted as a function of Trolox concentration. The unit of total antioxidant activity (TAA) was defined as the concentration of Trolox having equivalent antioxidant activity expressed as µmol/g sample extracts on dry matter basis.

RESULTS AND DISCUSSION

Total viable counts of bacteria in locust and velvet beans during fermentation

Table 1 showed the microbial load of the fermented locust beans and velvet beans that there were variations in total microbial counts based on cooking time and duration of fermentation. For fermented locust beans, cooking reduced microbial loads for samples fermented at both 12 h and 24 h. However, highest is 0.95×10^9 cfu/ml at 24 h of fermentation and 4.9×10^8 cfu/ml was observed in locust beans cooked for 30 min and fermented for 36 h. Increase in microbial load was observed at 12 h and 24 h of fermentation for uncooked fermented velvet and fermented velvet beans cooked for 15 min. Lowest microbial load of 0.6×10^9 cfu/ml was observed in velvet beans cooked for 30 min and fermented for 24 h.

Table 1 Total microbial counts of locust and velvet beans fermented at 35°C.

Samples	12 h (cfu/ml)	24 h (cfu/ml)	36 h (cfu/ml)
LBO	1.74×10^9	1.13×10^9	4.25×10^8
LB15	1.3×10^8	0.8×10^9	1.15×10^8
LB30	0.6×10^8	0.95×10^9	4.9×10^8
VBO	1.04×10^9	4.25×10^8	1.5×10^8
VB15	0.7×10^8	4.75×10^8	1.5×10^8
VB30	3.8×10^8	0.6×10^9	1.2×10^9

Values are expressed as means ± standard deviation on a dry weight basis.

Legends: LBO – Locust beans cooked for 0 min, LB15 – Locust beans cooked for 15 min, LB30 – Locust beans cooked for 30 min, VBO – Velvet beans cooked for 0 min, VB15 – Velvet beans cooked for 15 min, VB30 – Velvet beans cooked for 30 min.

From the revelation of the result in Table 1, there was initial increase in the total viable count of bacteria in some of the samples both pressure cooked and uncooked within the 24 h of fermentation after which it started fluctuating. This could be as a result of the change in the growth of microorganism as fermentation progressed. Similar results were observed during fermentation of African oil bean seeds as reported by **Enujiugha et al., (2008)**. **Babalola and Giwa, (2012)** reported increase in microbial load of cooked soybeans after 24 h of fermentation. This observation is also in agreement with the results obtained by **David and Aderibigbe, (2010)** during microbiological examination of *ogiri*. The decline and fluctuation in microbial loads of some samples could be as a result of depletion of nutrient in the fermenting medium which led to competition for survival among the organisms. On the other hand, initial increase in microbial load at the start of fermentation could be associated with the fact that, the microbes are in there stationary phase, thereby, the available nutrients encouraged their growth (**Babalola and Giwa, 2012**).

Physicochemical analyses of condiments from locust and velvet beans

The pH of the fermented legumes (cooked and uncooked) increased as cooking time and hour of fermentation increased. Samples cooked for 30 min had the highest value of 6.5 and 6.05 for locust beans and velvet beans at 12 h of fermentation respectively. Similar trend was observed at 24 h and 36 h of fermentation. In all instances, for both fermented legumes, high level of pH was observed at cooking time of 30 min (Table 2).

Results of total titratable acidity of fermented locust beans and velvet beans are show in Table 2. In all the samples, it was observed that TTA decreased as hour of fermentation increased. Total titratable acidity level of uncooked fermented locust beans decreased from 8.6 to 5.04 at 36 h of fermentation. Similar trend was observed for uncooked fermented velvet beans which have a value of 6.87 % at 36 h of fermentation. At 12 h of fermentation, fermented locust beans cooked for 15 min had the highest titratable acidity of 15.42 %.

As shown in Table 2, it was observed that there was increase in pH value of locust beans and velvet beans during the course of fermentation. This observation is similar to the finding of **Egounlety, (2003)** who noticed increase in the level of pH of *Mucuna* tempe and *Mucuna* condiment after 48 h of fermentation and the

increase was attributed to protein hydrolysis during fermentation. Increase in pH during fermentation of protein-rich oil seeds has also been reported by several authors (**David and Aderibigbe, 2010; Babalola and Giwa, 2012**). Increase in pH during fermentation has also been attributed to proteolytic activities and the release of ammonia following the utilization of amino acids by microorganisms involved in the fermentation (**Sarkar et al., 1993**). Ammonia is mainly responsible for the characteristic pungent smell that usually accompanies most vegetative protein fermentation. The Table also revealed that there was decrease in titratable acidity (TTA) of samples of condiments from fermented locust and velvet beans, as fermentation progressed. This is an indication that less of organic acids production and more of proteinase activity occurred during fermentation (**Nout, 1994**).

Table 2 Physicochemical parameters [pH and total titratable acidity (TTA)] of locust and velvet beans fermented at 35 °C

Samples	12h	24h	30h	36h
pH				
LBO	5.92 ± 0.02 ^b	6.16 ± 0.01 ^c	6.48 ± 0.03 ^c	6.83 ± 0.03 ^c
LB15	6.20 ± 0.00 ^d	6.30 ± 0.02 ^d	6.52 ± 0.01 ^c	6.80 ± 0.00 ^c
LB30	6.50 ± 0.04 ^c	7.10 ± 0.00 ^c	7.18 ± 0.01 ^d	7.30 ± 0.00 ^d
VBO	5.33 ± 0.03 ^a	5.40 ± 0.02 ^a	5.54 ± 0.00 ^a	5.72 ± 0.00 ^a
VB15	6.05 ± 0.05 ^c	6.05 ± 0.00 ^b	6.13 ± 0.01 ^b	6.25 ± 0.00 ^b
VB30	6.05 ± 0.00 ^c	6.08 ± 0.00 ^b	6.14 ± 0.02 ^b	6.20 ± 0.00 ^b
Total titratable acidity				
LBO	8.60 ± 0.04 ^b	7.85 ± 0.05 ^c	6.52 ± 0.02 ^b	5.04 ± 0.02 ^b
LB15	15.42 ± 0.04 ^e	8.37 ± 0.03 ^d	7.13 ± 0.01 ^c	6.17 ± 0.01 ^c
LB30	7.32 ± 0.02 ^a	5.33 ± 0.03 ^a	4.67 ± 0.03 ^a	3.65 ± 0.03 ^a
VBO	7.06 ± 0.01 ^a	7.02 ± 0.02 ^b	6.92 ± 0.01 ^c	6.87 ± 0.03 ^d
VB15	9.12 ± 0.01 ^c	7.95 ± 0.05 ^c	7.67 ± 0.03 ^d	7.32 ± 0.02 ^e
VB30	10.12 ± 0.02 ^d	9.02 ± 0.02 ^e	7.97 ± 0.01 ^{de}	6.31 ± 0.01 ^c

Values are expressed as means ± standard deviation on a dry weight basis. Means of triplicate determinations ± S.D.

Means with different superscripts on the same column are significantly different at p ≤ 0.05

Legends: LBO – Locust beans cooked for 0 min, LB15 – Locust beans cooked for 15 min, LB30 – Locust beans cooked for 30 min, VBO – Velvet beans cooked for 0 min, VB15 – Velvet beans cooked for 15 min, VB30 – Velvet beans cooked for 30 min.

Phytochemical composition of condiments from fermented locust and velvet beans

The results of the effect of increase in cooking duration and fermentation with *Bacillus subtilis*, on the levels of some anti-nutritional factors of African locust beans and velvet beans are given in Table 3. Oxalate levels of the samples were significantly different (p ≤ 0.05) with uncooked fermented locust beans having the highest value of 0.69 mg/g. There was no significant difference (p ≥ 0.05) in the saponin level of fermented locust beans and fermented velvet beans at the same cooking time. Phytate level of fermented locust beans decreased from 28.83 mg/g to 25.42 mg/g at 30 min cooking time. Similar trend was observed for phytate level of fermented velvet beans. The values obtained for trypsin inhibitor were all significantly different (p ≤ 0.05) with the exception of uncooked fermented locust beans and locust fermented after 15 min of cooking. There was decrease in the level of tannin as cooking time progressed, tannin decreased from 4.46 mg/100 g to 3.49 mg/100 g in fermented locust beans and 8.46 mg/100 g to 8.08 mg/100g in fermented velvet beans.

The level of phytochemical constituent in locust beans and velvet beans, as influenced by cooking and fermentation was shown (Table 3 to decreased significantly (p ≤ 0.05) with increase in the duration of cooking. This finding supports the report of **Ezeagu et al., (2003)**. Some microorganisms have been reported to possess enzymes that are capable of hydrolyzing anti-nutrients (**Gabriel et al., 2004**). Oxalate content of the samples was influenced by cooking as the values lowered at specified cooking durations. This observation is in agreement with the finding of **Iorgyer et al., (2009)** on reduction of oxalate of pigeon pea seeds. The level of phytate in fermented locust and velvet beans was found to reduce with increase in the duration of cooking and fermentation. Several authors have reported similar observation in this regard (**Siddhuraju et al., 1996; Mugendi et al., 2010**) noticed that roasting, germination and fermentation reduced level of phytate in *Mucuna* beans.

Table 3 Phytochemical composition of condiments from locust and velvet beans fermented at 35°C.

Samples	Oxalate (mg/g)	Saponin (mg/100 g)	Phytate (mg/g)	Trypsin Inhibitor Activity (mg/g)	Tannin (mg/100 g)
LBO	0.69±0.00 ^a	0.91±0.00 ^a	28.83±0.02 ^b	3.60±0.02 ^a	4.46±0.00 ^c
LB15	0.58±0.00 ^d	0.80±0.01 ^b	26.22±0.16 ^c	3.61±0.01 ^a	3.95±0.01 ^d
LB30	0.55±0.01 ^e	0.72±0.00 ^c	25.42±0.12 ^d	3.05±0.04 ^b	3.49±0.01 ^e
VBO	0.68±0.01 ^b	0.90±0.02 ^a	31.93±0.21 ^a	2.72±0.03 ^c	8.46±0.00 ^a
VB15	0.62±0.00 ^c	0.80±0.01 ^b	25.10±0.38 ^c	1.70±0.05 ^d	8.17±0.02 ^b
VB30	0.55±0.00 ^e	0.73±0.00 ^c	25.38±0.17 ^d	1.20±0.02 ^e	8.08±0.0 ^b

Values are expressed as means ± standard deviation on a dry weight basis. Means of triplicate determinations ± S.D.

Means with different superscripts on the same column are significantly different at p≤0.05

Legends: LBO – Locust beans cooked for 0 min, LB15 – Locust beans cooked for 15 min, LB30 – Locust beans cooked for 30 min, VBO – Velvet beans cooked for 0 min, VB15 – Velvet beans cooked for 15 min, VB30 – Velvet beans cooked for 30 min.

Low levels of saponins are present in the samples and there was slight reduction after cooking and fermentation. **Nwaoguikpe et al., (2011)** observed reduction in the concentration of saponins in velvet beans after soaking and cooking. Saponins are recently shown to have hypocholesterolemic as well as anti-carcinogenic effects. Trypsin inhibitor activity (TIA) levels of both condiments from locust beans and velvet beans reduced after cooking and fermentation. The loss in TIA content during processing can be attributed to leaching during soaking, by heat treatment during boiling and also by the action of microorganisms during fermentation **Esenwah and Ikenebomeh, (2008)** has also observed in the fermentation of African locust beans. There was significant (p≤0.05) decrease in tannin level of the samples. Loss of tannin may be due to its solubility in water and its sensitivity to heat during boiling (**Esenwah. and Ikenebomeh, 2008**), since later report indicated that processing methods such as soaking, boiling and fermentation lowered the tannin contents of the foods which may also be due to the thermal degradation and denaturation. Since the seed coats are usually removed by soaking prior to consumption, the tannins in *Mucuna* beans are of little significance from the nutritional point of view (**Mugendi et al., 2010**). Tannins are known to possess health benefits, wherein they are 15–30 time more efficient in radical quenching activity than Trolox and other simple phenolics (**Gallegos-Infante et al., 2007**).

Antioxidant composition of condiments from locust and velvet beans

Figure 1 and 2 shows the antioxidant properties of condiments produced from fermented locust beans and velvet beans. The total phenolic content increased as cooking time increased for all samples of fermented locust beans and velvet beans (Figure 1). It also revealed that there was decrease in total flavonoid content for fermented locust beans after 15 min of cooking, this later increased slightly at 30 min of cooking. The level of flavonoid in fermented velvet beans was constant at both 15 min and 30 min of cooking. The vitamin C content of fermented locust beans was uncovered to increase after cooking but there was decrease in the level of vitamin C in fermented velvet beans when subjected to cooking. It was also showed that FRAP of fermented locust beans increased slightly at 15 min of cooking. However, the level of FRAP (Ferric Reduction Antioxidant Power) of fermented velvet beans reduced steadily as cooking progressed. Reduction was observed in the level ABTS for fermented locust beans after cooking but rose slightly for fermented velvet beans after cooking for 15 min. The free radical (DPPH) scavenging activity level of fermented locust beans was raised at 15 min of cooking unlike fermented velvet beans which reduced at the same cooking interval (Figure 2).

As it is made known in Figure 1, the total phenolic content (TPC) of locust beans and velvet beans increased with increase in the duration of cooking and fermentation. The increase can be attributed to duration of the cooking which enhanced diffusion of phenols from seed coats to cooking water and from there to cotyledons. According to **Singleton et al., (1999)** a minor amount of phenols can be explained by a lixiviation phenomenon that drives phenols into the cooking water. This process is a function of temperature (high temperature leads to an increase in lixiviation), and will promote diffusion of phenols into cotyledons. This phenomenon might have occurred in locust and velvet beans during cooking for both 15 min and 30 min. Similar increase in phenols was observed after cooking of common bean (*Phaseolus vulgaris* L.) cultivars (**Gallegos-Infante et al., 2007**). The presence of high amount of phenols indicates that the condiments could act as anti-inflammatory, anti-clotting, antioxidant, immune enhancers and hormone modulators (**Okwu and Omodamiro, 2005**).

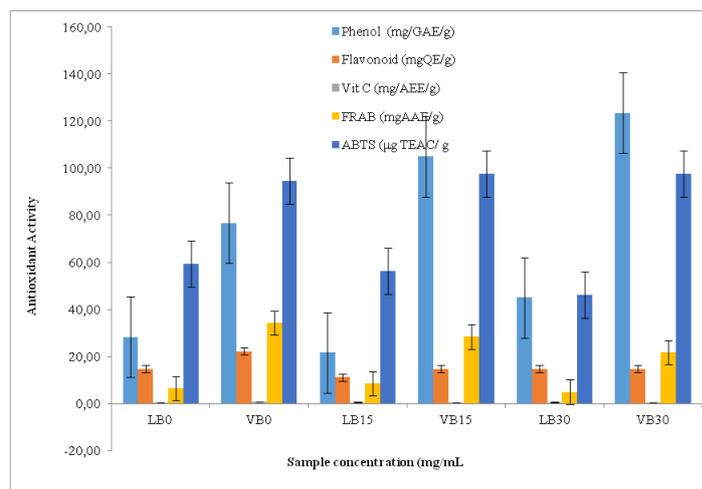


Figure 1 Antioxidant activity of condiments from locust and velvet beans fermented at 35°C.

Legends:
 LBO – Locust beans cooked for 0 min,
 LB15 – Locust beans cooked for 15 min,
 LB30 – Locust beans cooked for 30 min,
 VBO – Velvet beans cooked for 0 min,
 VB15 – Velvet beans cooked for 15 min,
 VB30 – Velvet beans cooked for 30 min.

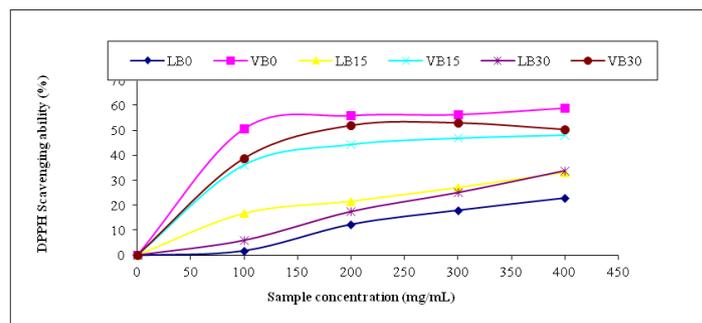


Figure 2 2-diphenyl-picryl-hydrazyl (DPPH) scavenging activity of condiments from locust and velvet beans fermented at 35 °C.

Values are expressed in % scavenging activity
Legends:
 LBO – Locust beans cooked for 0 min,
 LB15 – Locust beans cooked for 15 min,
 LB30 – Locust beans cooked for 30 min,
 VBO – Velvet beans cooked for 0 min,
 VB15 – Velvet beans cooked for 15 min,
 VB30 – Velvet beans cooked for 30 min

There was reduction in total flavonoid content (TFC) of fermented locust beans and velvet beans as shown in Figure 1. Similar decrease in flavonoid content of locust beans was observed by **Enujiugha, (2010)** who studied effects of solvent extraction and heat treatment methods on phenolics components of African locust bean seeds. Notably, flavonoid concentrations were affected by cooking; this confirms the work of **Gallegos-Infante et al., (2007)** which reported that flavonoids are destroyed by heat processing methods like drying, roasting and boiling. Flavonoids are widely distributed group of polyphenolic compounds,

characterized by a common benzopyrone ring structure that has been reported to act as antioxidants in various biological systems. The biological functions of flavonoids apart from its antioxidant properties include protection against allergies, inflammation, free radicals, platelet aggregation, microbes, ulcers, viruses and tumors (Okwu and Omodamiro, 2005). Flavonoids reduce cancer by interfering with estrogen synthetase, an enzyme that binds estrogen to receptors in several organs (Okwu and Omodamiro, 2005).

There was reduction in the level of vitamin C of both cooked, fermented locust and velvet beans as shown. The decrease in the level of vitamin C of cooked fermented velvet beans can be linked to oxidation and destruction of vitamin C during cooking. Also, high levels of phenolics in the fermented beans decrease the availability of vitamin and minerals (Mugendi et al., 2010). Vitamin C being an iron absorption enhancer is known to counteract the inhibitory effects of phytate in intestine (Siegenberg et al., 1991). Ferric Reducing Property (FRAP) assay depends on the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant (antioxidants or other reducing agents) at low pH. As shown in Figure 4, there was reduction in FRAP of both beans as cooking progressed and this can be linked to increase in phenols which generally reduced antioxidant power of the condiments (Singleton et al., 1999). Similar reason can be adduced to reduction in ABTS Radical Scavenging Ability after cooking as shown. The increase in phenols as cooking progressed has affected the overall anti-oxidant properties of both cooked fermented locust beans and velvet beans.

The mechanism of DPPH radical scavenging assay is based on the reduction of DPPH* solution in the presence of a hydrogen donation antioxidant, inducing the formation of non-radical form DPPH-H (Yang et al., 2008). It is interesting to note that DPPH Radical Scavenging Activity is directly linked to the concentration of the samples, because higher levels of extracts show higher effect in free radical trapping. Figure 2 revealed higher percentage scavenging ability was detected in cooked fermented, sample which shows that at longer cooking, locust and velvet beans have high abilities for trapping free radicals. However, the fall in percentage radical scavenging ability (30 min of cooking) as also observed for soy bean oil (Jalali Mousavi et al., 2015) for both beans can be linked to diffusion of phenols to cooking water and cotyledon and the formation of complex phenol-protein, which, is not an active component to produce antioxidant effects. Similar observation was made by Gallegos-Infante et al. (2007) after analysis of scavenging ability of some cooked cultivars of common bean (*Phaseolus vulgaris* L).

CONCLUSION

This research work has shown that the use of *Bacillus subtilis* resulted in improving nutritional quality of condiments from locust beans and velvet beans. It was discovered that the condiment possessed antioxidant properties which were found to decrease due to processing methods. Also, the presence of some phytochemicals and the antioxidant properties demonstrated by fermented velvet bean and locust beans may be an indication that the legumes can be employed as functional foods.

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest on the research work.

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