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Physicochemical and nutritional properties of *Mucuna pruriens* and *Parkia biglobosa* subjected to controlled fermentation

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Abstract

Mucuna pruriens (velvet bean) and *Parkia biglobosa* (locust bean) were dehulled and the beans were 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 beans were then fermented using mixture of starter cultures of *Bacillus subtilis* 7A and BCD 333 for 36 h at 30^oC. The results of the physicochemical properties of the fermented beans include, pH (6.40–7.60), total titratable acidity (7.01-22.72), protein (25.88%-35.35%), fat (8.45%-37.91%), fibre (2.16%-14.30%) and ash content (0.61%-3.56%). The results of the mineral composition showed that fermented locust bean possessed values that were significantly higher than velvet bean in magnesium (189.00 mg/100g-247.67 mg/100g), sodium (3.22 mg/100g-71.31mg/100g), iron (3.62 mg/100g-8.61 mg/100g) and calcium (179.33 mg/100g-292.33mg/100g), while velvet bean was found to be richer in potassium (471.33 mg/100g-1002.00 mg/100g). Furthermore, it was observed that aspartame (7.96 g/mg-10.12 g/mg) and glutamate (13.78 g/mg-27.79 g/mg) were abundant in the fermented beans and appreciable amount of essential amino acids with the exception of methionine. The result of the sensory showed that there was no significant difference in uncooked condiments from both fermented locust beans and velvet beans, which may be an indication that fermented *M. pruriens* may serve as an alternative to fermented *P. biglobosa.*

Introduction

Food legumes are major sources of proteins in the diets of many people in developing countries. In fact, the high cost of animal protein has brought about interest towards several leguminous seed as potential sources of vegetable proteins for human food and livestock feed. Since legume seeds are important sources of proteins, there has been a worldwide interest in searching for potential utilization of unconventional legumes (Siddhuraju *et al*., 1996). The prevailing population pressure in developing countries has resulted in the exploitation of under-utilized nutritious plant products with aesthetic and organoleptic appeal in the daily diet (Enujiugha, 2003). The common edible portions of most under-utilized plants are the seeds, which in some cases are cooked or roasted and eaten directly as snack foods.

Mucuna pruriens is a tropical legume known as velvet beans consumed and promoted by smallholder farmers in Africa, South America and South Asia as a green manure or a cover crop (Ezeagu *et al*., 2003). It commonly produces 200 to 600 kg of seeds per hectare which are very rich in protein (24%-35%) and its digestibility is comparable to that of other pulses, such as soybean and lima bean (Bressani, 2002; Gurumoorthi *et al*., 2003). Despite its potential, velvet bean is poorly adopted in agricultural systems due to the presence of antinutritional compounds which lower the nutrient value of grain legumes, reduce food intake and nutrient utilization in animals (Capo-chichi *et al*., 2003).

Parkia biglobosa, commonly known as African locust bean is a perennial tree legume grown in the savannah region of West Africa (Campbell-platt, 1980). Locust bean *(Parkia biglobosa)* is fermented and sold locally in Nigeria as mashed beans "Iru pete" and unmashed "iru woro" (whole beans) (Ladokun and Adejumo, 2013). The most important use of locust bean is found in its seeds which are rich in protein, lysine, lipids, carbohydrate, vitamin B_2, and contain easily digestible calcium (Akande *et al*., 2010).

Bacillus subtilis, a strong proteolytic bacterium is mainly used in alkaline fermentation of legumes. It causes biochemical changes in beans by hydrolysis of proteins and metabolism of resultant amino acids leading to increase in pH and flavour development (Owens *et al*., 1997). In legume processing, combinations of two or more methods are used to

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effectively remove antinutritional compounds (Sathe and Salunkhe, 1984). Fermentation is one of the oldest methods used in improving the nutritional quality of legumes. Production of condiment from oil seeds such as African locust bean, melon seed, castor oil seed and soybean have been reported (Egounlety and Aworh, 2000; Egounlety, 2003). This research was carried out to compare the quality of condiment obtained from fermentation of velvet and locust bean using *Bacillus subtilis* 7A and BCD 333 as starter cultures.

Materials and Methods

Materials

Velvet beans *(Mucuna pruriens)* and locust beans *(Parkia biglobosa)* used in this study were purchased from International Institute of Tropical Agriculture (IITA) in Ibadan, Nigeria and local market in Akure, Nigeria respectively. All reagents used for the analyses were of analytical grade.

Harvest of microbial cells used for fermentation

Bacillus subtilis 7A and BCD 333 cultures 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* isolates were separately activated overnight in nutrient agar and harvested in graduated sterilized Eppendorf tubes of 5 ml using a micro centrifuge (Stuart microfuge SRFC1 10000 X rpm) at 6000 rpm for 5 min. 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 and centrifugation was repeated at 6000 rpm for 3 min to obtain pure cells. The harvested cells were maintained on ice prior to usage.

Production of Fermented Locust Beans and Velvet beans

Parkia biglobosa were sorted to remove dirt and foreign materials, cooked for 2 h and dehulled using mortar and pestle. *Mucuna pruriens* were sorted, soaked in hot water for 10 min and hand dehulled. The dehulled seeds 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 un-pressure cooked dehulled samples were soaked in 10% hypochlorite for sterilization. Samples were separately transferred into sterile foil plate and inoculated with Bacillus subtilis 7A and BCD 333 (about 10^8 CFU/ml) under aseptic conditions. Samples were individually wrapped and allowed to ferment for 36 h at 30°C. The resulting fermented seeds were dried in an oven for 6 h at 60°C, milled and stored at 4ºC for further analyses (Gabriel *et al*., 2004).

Determination of pH

The pH of samples were determined at every 12 h interval throughout the 36 h fermentation time. This was done by weighing 2 g of the fermenting mass, homogenized in a blender with 20 ml of distilled water and the pH of the homogenate was taken using a pH meter (Model 401).

Determination of total titratable acidity

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 0.1M NaOH using phenolphthalein as indicator. The titre value was then used to calculate the titratable acidity as percentage lactic acid using Association of Official Analytical Chemists (AOAC, 2005).

Proximate composition

The proximate analysis (moisture, total ash, crude fat, crude protein, and crude fibre of the samples were carried out in triplicate (AOAC, 2005).

Determination of mineral content

The mineral content of the samples were determined using Energy dispersive X-ray fluorescence spectroscopy. The sample was placed on a rotating tray; a high power X-ray tube irradiates a metal disc causing it to emit its own characteristic radiation lines. This fluorescent radiation excites the samples in the tray and energies were emitted. The spectra are shown on a detector which is capable of separating and measuring the different energies of the characteristic radiation emitted from the sample to determine the elements present. How much of a particular element present is determined by measuring the intensity of the emitted energies (Jerkins, 2000).

Determination of amino acid profile

Amino acid profile was determined using automated amino acid analyzer (Technicon Sequential Multi–sample Analyzer, TSM) (Benitez, 1989; AOAC 2005).

Sensory evaluation

Sensory evaluation of the condiments was conducted by a panel of 10 untrained judges who are

Table 1. pH of Locust beans and velvet beans during fermentation

Samples	12 h	24 h	36 h
LB0	6.02±0.01[d]	6.31±0.01[c]	6.60±0.02[c]
LB15	6.32±0.02[b]	6.51±0.01[b]	6.72±0.02[b]
LB30	6.40±0.01[a]	7.42±0.02[a]	7.61±0.01[a]
VB0	5.36±0.01[e]	5.45±0.01[f]	5.54±0.01[f]
VB15	6.06±0.01[c]	6.11±0.01[e]	6.17±0.02[e]
VB30	6.06±0.01[c]	6.17±0.02[d]	6.36±0.01[d]

Means with different superscripts on the same column are significantly different at p≤0.05
LB0 – un-pressure cooked locust beans, LB15 – Locust beans pressure cooked for 15 min , LB30 –Locust beans pressure cooked for 30 min, VB0 – un-pressure cooked velvet beans, VB15 – Velvet beans pressure cooked for 15 min, VB30 – Velvet beans pressure cooked for 30 min.

regular consumers of locally produced condiments. The judges scored the samples for colour, taste, texture, flavour and general acceptability using a 9-point hedonic scale ranging from 1 = dislike extremely and 9 = like extremely (Solomakos et al., 2008).

Statistical Analysis

The experiments were determined in duplicates while means ± SD were calculated from triplicate determinations. Statistical analysis was performed using the Statistical Package for Social Sciences package. Data were subjected to analysis of variance (ANOVA). Comparison of means was carried out by Duncan's multiple range test (Steel and Torrie, 1980).

Results and Discussion

pH of locust and velvet beans during fermentation

The pH of the fermented beans are presented in Table 1. There was increase in pH value of locust beans (6.01-7.60) and velvet beans (5.35-6.35), while the pH of locust bean was found to be higher than that of velvet bean 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. Increase in pH during fermentation of protein-rich oil seeds have also been reported by several authors (Aderibigbe and Adebayo, 2002; David and Aderibigbe, 2010; Babalola and Giwa, 2012). This has 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. There were reductions in titratable acidity (TTA) of samples of condiments from fermented locust and velvet beans as fermentation progressed (result not shown). This is an indication that less of organic acids production and more of proteinase activity occurred during fermentation (Nout, 1994). It could also be due to the action of microbial enzymes on substrate proteins, carbohydrates and lipids. Some *Bacillus* species have been reported to possess the ability to produce enzymes such as proteases, that breakdown protein to amino acids thus making the medium to become alkaline due to the presence of amino acids (Oyarekua, 2011).

Proximate Composition of Condiments from fermented Locust Beans and Velvet Beans

The protein content of the fermented locust (27.72%-38.22%) and velvet beans (28.38%-32.55%) showed that the value increased with increase in cooking time and protein content of locust bean is significantly higher than that of velvet bean (Table 2). This may imply that cooking of legumes before use in human diet improves the protein quality by either destroying or inactivating heat-labile antinutrients (Mubarak, 2005).The value obtained for protein content of locust beans fermented with *B. subtilis* for 36 h (35.35%), was found to be higher than 32.90% and 19.90% for locust beans and melon fermented naturally for 72 h respectively (Omafuvbe *et al*., 2004). *P. biglobosa* is a major source of plant protein and fat and also a suggestive nutrient in combating protein deficiency (Yusuf *et al.*, 2007). The fat content

Table 2. Proximate composition (%) of fermented locust beans and velvet beans (dry weight basis)

Samples	Moisture Content	Crude Protein	Fat Content	Ash Content	Crude Fibre Content	Carbohydrate
LB0	10.21± 0.08^{a}	29.77± 0.69^{c}	42.22± 1.00^{a}	3.97± 0.04^{a}	15.93± 0.15^{a}	8.10± 0.66^{d}
LB15	6.63 ± 0.07^{d}	27.72± 0.05^{e}	33.24± 0.11^{c}	0.65± 0.02^{f}	14.31± 0.19^{b}	24.08±0.28^{b}
LB30	7.49± 0.04^{c}	38.22± 0.09^{a}	36.12± 0.47^{b}	0.93± 0.02^{e}	8.00± 0.02^{c}	16.71± 0.42^{c}
VB0	7.79± 0.30^{b}	28.38± 0.09^{d}	11.41± 0.07^{d}	2.31± 0.02^{d}	6.34± 0.01^{d}	51.56± 0.11^{a}
VB15	8.03± 0.07^{b}	29.47± 0.05^{c}	11.36± 0.14^{d}	2.94± 0.01^{c}	6.32± 0.07^{d}	51.23± 2.29^{a}
VB30	6.82± 0.10^{d}	32.55± 0.26^{b}	9.07± 0.37^{e}	3.36± 0.06^{b}	2.32± 0.04^{e}	53.04± 0.58^{a}

Means with different superscripts on the same column are significantly different at p≤0.05 LB0 – un-pressure cooked locust beans, LB15 – Locust beans pressure cooked for 15 min, LB30 – Locust beans pressure cooked for 30 min, VB0 – un-pressure cooked velvet beans uncooked, VB15 – Velvet beans pressure cooked for 15 min, VB30 – Velvet beans pressure cooked for 30 min

of the locust bean (32.24%-42.22%) was found to be higher than that of ‘iru woro” (22.80%), an unmashed fermented locust bean and lower than that of “iru pete” (49.93%), a mashed fermented locust beans (Ladokun and Adejumo, 2013). However, it was observed that fermented velvet bean (9.07%-11.41%) possessed fat content that were significantly lower than that of fermented locust bean. This may be an indication that fermented velvet bean may be employed when product with lesser fat content is preferred.

The decrease in ash content of pressure cooked fermented locust beans (0.65%-3.97%) when compared to the un-pressure cooked sample (2.31%) follows the same trend with previous report (Esenwah and Ikenebomeh, 2008). This may be due to leaching of soluble inorganic salts into the cooking water during processing (Osman, 2007; Ogbonnaya et al., 2010) or the fermenting microflora used it for their metabolism (Reebe *et al*., 2000). It however falls within the 2.9%-4.4% range reported for other *Mucuna varieties* (Vijayakumari *et al*., 1996). Increase in ash content can be attributed to destruction of antinutrients especially phytate which is thought to be responsible for impairment of mineral bio-availability (Osman, 2007). The crude fibre content of fermented locust beans (8.00%-15.93%) and velvet bean (2.32%-6.34%) were found to decrease with increase in cooking time. Decrease in crude fibre with increase in duration of cooking time of mucuna spp agrees with previous report (Akinmutimi and Ukpabi, 2008). This could be as result of the degradation of the fibre by fermenting microbes (Babalola and Giwa, 2012). In addition, boiling and dehulling of the locust bean seed may be responsible for the reduction recorded (Enujiugha, 2003). During processing, it was observed that the water used to boil locust bean was more viscous than it was at the beginning of the process. This may be due to the presence of mucilaginous materials in the water which would explain in part the reduction of the crude fibre content of the locust bean seed .The carbohydrate content of the fermented locust beans and velvet beans increased with duration of cooking. Similar trend was observed by Arisa *et al.* (2010) who noticed increase in the level of carbohydrate of *Mucuna flagellipes* after boiling for 90 min.

Mineral Composition of Condiments from Locust and Velvet Beans

Magnesium (247.67mg/100g-189.00mg/100g), sodium (71.31mg/100g), zinc (3.24 mg/100g-0.05 mg/100g) and calcium (292.00 mg/100g-179.00 mg/100g) were found to reduce in the pressure cooked samples of locust bean (Table 3). Significant decreases in the levels of phosphorus, iron and selenium of Mucuna seeds as the boiling time increased have been documented (Tuleun *et al*., 2009). Also, Olaofe and Sanni (1998) reported that all processing methods reduced magnesium, calcium and sodium. Aletor and Ojo (1989) observed decrease in potassium, magnesium, sodium and phosphorus levels of cooked samples of soybeans and lima beans. Oladunmoye (2007) reported that during processing, some of the minerals may be leached into the growth medium. However, the samples were found to contain substantial level of potassium, magnesium and calcium which support the finding of Elemo et al. (2011) who observed that processed African locust beans is a rich source of phosphorus, magnesium, potassium and calcium. Calcium in conjunction with phosphorus, magnesium, manganese, vitamins A, C, and D are involved in bone formation (Ogunlade *et al*., 2005).

Amino acid composition of condiments from locust

Table 3. Mineral composition of condiments from locust and velvet beans (mg/100 g)

Samples	Magnesium	Copper	Iron	Potassium	Sodium	Zinc	Calcium
LB0	247.67±1.52[a]	6.02±0.02[b]	4.08±0.01[c]	92.50±0.91[f]	71.31±10.45[a]	3.24±0.08[a]	292.33±4.16[a]
LB15	164.33±0.57	14.59±0.06[a]	3.62±0.60[d]	130.63±2.08[e]	3.74±0.00[c]	0.05±0.02[d]	179.33±6.02[c]
LB30	189.00±1.73[b]	1.67±0.00[c]	8.61±0.04[a]	171.43±1.81[d]	3.22±0.10[c]	0.61±0.80[c]	214.00±4.58[b]
VB0	97.00±0.00[f]	1.37±0.02[e]	5.01±0.07[b]	471.33±10.06[c]	16.39±1.22[b]	0.69±0.01[f]	36.78±0.52[d]
VB15	127.33±1.52[e]	1.52±0.02[d]	4.45±0.04[c]	1002.00±13.22[a]	1.10±0.08[c]	1.02±0.08[b]	27.97±0.54[e]
VB30	136.33±0.57[d]	1.33±0.01[e]	4.36±0.04[c]	987.67±3.05[b]	0.48±0.05[c]	0.16±0.05[e]	39.24±0.43[d]

Means with different superscripts on the same column are significantly different at p≤ 0.05 LB0 – un-pressure cooked locust beans, LB15 – Locust beans pressure cooked for 15 min, LB30 – Locust beans pressure cooked for 30 min, VB0 – un-pressure cooked velvet beans uncooked, VB15 – Velvet beans pressure cooked for 15 min, VB30 – Velvet beans pressure cooked for 30 min

Table 4. Essential amino acid of condiments from locust and velvet beans

Amino Acid (g/mg)	LB0	LB15	LB30	VB0	VB15	VB30	FAO/WHO(1991)
Leucine	4.86	6.77	9.98	7.59	7.39	8.71	4.8
Isoleucine	2.87	3.04	5.51	4.57	2.88	5.17	4.2
Lysine	4.05	6.63	7.06	4.38	5.95	6.91	-
Threonine	3.29	3.67	5.64	3.34	2.80	5.64	2.6
Methionine	0.82	1.55	1.53	1.52	1.65	1.62	4.2
Valine	2.10	5.17	6.39	4.66	4.41	5.80	2.8
Phenylalanine	7.25	4.14	6.46	5.79	4.03	5.48	-
Histidine	1.83	2.20	7.63	2.69	2.13	6.69	2.4
Arginine	3.12	7.02	6.86	9.80	7.29	6.16	2.00
TEEA	30.19	40.19	55.56	44.34	43.53	50.58	-

TEAA – Total Essential Amino Acids
LB0 – un-pressure cooked locust beans, LB15 – Locust beans pressure cooked for 15 min , LB30 –Locust beans pressure cooked for 30 min, VB0 – un-pressure cooked velvet beans uncooked, VB15 – Velvet beans pressure cooked for 15 min, VB30 – Velvet beans pressure cooked for 30 min.

beans and velvet beans

The essential amino acid profile of condiments from fermented locust beans and velvet beans is presented on Table 4. It was observed that processed locust beans contain appreciable level of essential amino acids with the exception of methionine which is limiting in legumes. Similar finding was reported by Elemo *et al.* (2011) who observed meager level of methionine in processed African locust beans. Both uncooked and cooked fermented beans were found to be rich in essential amino acids, most especially, lysine which makes it a useful supplement for cereal grains that are generally low in lysine (Iyayi and Taiwo, 2003). Leucine, threonine, phenylalanine, valine, histidine and arginine of condiments from fermented locust and velvet beans compared favourably with the FAO/WHO (1991) requirement pattern. Aspartame and glutamate were predominant in fermented locust and velvet beans and this is consistent to previous reports (Siddhuraju *et al.*, 2000; Ogungbenle *et al.*, 2005).

Sensory evaluation of condiments from locust and velvet beans

The result of the sensory evaluation for the fermented locust and velvet bean is shown on Table

Table 5. Sensory evaluation of condiments from locust and velvet beans

Samples	Colour	Taste	Texture	Flavour	General Acceptability
LB0	$7.80±1.03^{a}$	$6.30±1.63^{a}$	$6.30±1.25^{ab}$	$7.00±1.82^{a}$	$7.10±1.19^{a}$
LB15	$6.90±2.33^{a}$	$6.50±2.59^{a}$	$6.00±2.10^{ab}$	$5.00±2.90^{a}$	$6.00±2.26^{ab}$
LB30	$6.60±1.57^{ab}$	$5.80±1.81^{ab}$	$6.20±2.09^{ab}$	$5.40±2.27^{a}$	$6.10±1.96^{ab}$
VB0	$7.56±0.64^{a}$	$6.20±1.27^{ab}$	$6.55±1.03^{a}$	$6.45±1.72^{a}$	$6.55±1.03^{a}$
VB15	$5.30±1.60^{bc}$	$5.20±2.66^{ab}$	$5.15±1.81^{ab}$	$5.40 ±2.37^{a}$	$4.80±2.13^{b}$
VB30	$4.90±2.17^{c}$	$4.40±1.42^{b}$	$4.75±1.76^{b}$	$4.90±1.52^{a}$	$4.55±1.30^{b}$

Means with different superscripts on the same column are significantly different at p≤0.05. LB0 – un-pressure cooked locust beans; LB15 – Locust beans pressure cooked for 15 min,
LB30 – Locust beans pressure cooked for 30 min; VB0 – un-pressure cooked velvet beans uncooked, VB15 – Velvet beans pressure cooked for 15 min; VB30 – Velvet beans pressure cooked for 30 min.

5. There was no significant difference (p ≥0.05) in the colour of uncooked fermented locust and velvet beans, while the pressure cooked samples were significantly different. Both the uncooked (6.30) and 15 min pressure cooked (6.50) fermented locust bean were highly scored for taste, next were uncooked velvet bean (6.20), locust bean that was cooked for 30 min (5.80) and 15 min cooked velvet (5.20) while velvet bean that was cooked for 30 min (4.40) was scored below average. For general acceptability, both the uncooked locust and velvet bean were most preferred (6.55-7.10), followed by cooked locust bean (6.00-6.10) and cooked velvet bean (4.55-4.80). Amoa-Awua *et al.* (2006) reported that dawadawa produced from four isolates of *Bacillus subtilis* were highly preferred. Variation in scores obtained may be attributed to the texture of the fermented beans since the uncooked fermented samples were scored higher than all the pressure cooked samples.

Conclusion

Findings from this work revealed that locust and velvet bean fermented using *Bacillus subtilis* 7A and BCD 333 produced condiments that are rich in nutrients with the protein content of locust bean significantly higher than that of velvet bean. Considering the sensory evaluation, there was no significant difference in un-pressure cooked fermented locust and velvet beans and the samples were scored higher than the pressure cooked beans. This may be an indication that condiments produced from fermented *M. pruriens* may also be an alternative to fermented *P. biglobosa.*

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