

Comparative assessment of nutritional, colour and sensory properties of *Bacillus subtilis* BaAP3 - and spontaneously-fermented African locust bean (*Parkia biglobosa*) seeds

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Abstract

African locust bean (ALB) (*Parkia biglobosa*) seeds were subjected to spontaneous and *Bacillus subtilis* BaAP3 fermentation. The nutritional, colour and sensory properties of the samples were determined and comparatively assessed using standard methods. There was no significant difference in the moisture, dry matter, and ash contents of spontaneously fermented (SF) and *Bacillus subtilis* BaAP3 fermented (BSF) samples. The crude protein increased, while carbohydrate content reduced, significantly ($p < 0.05$) for both SF and BSF samples. The crude fat content for SF sample increased significantly ($p < 0.05$) from 44.36 to 53.91 g/100g, while there was no significant difference in crude fat values for BSF. There was no significant difference in the crude fibre contents of SF samples while that of BSF decreased significantly ($p < 0.05$). The calcium content reduced from 1075.00 to 1010.33 mg/kg for SF sample and increased from 1179 to 1221.33 mg/kg for BSF sample. The sodium and copper contents of both SF and BSF samples reduced significantly ($p < 0.05$) on fermentation. The sodium content reduced from 670.10 to 490.07 mg/kg for SF sample and from 740.20 to 600 mg/kg for BSF sample, while copper content reduced from 5.00 to 3.00 mg/kg for both samples. The magnesium, iron and zinc contents for SF and BSF samples were not significantly different. For potassium and phosphorus, the values increased for both samples. Potassium contents increased from 571.67 to 719 mg/kg and 580.00 to 631.67 mg/kg for SF and BSF samples, respectively. While phosphorus content increased from 2856.5 to 3244.4 mg/kg for SF sample and 2810.80 to 2970.17 mg/kg for BSF sample. There was no significant difference ($P > 0.05$) in the colour parameters for both samples and the values of the parameters suggested that the colour was grayish. The sensory properties of the fermented products indicated that BSF seed was preferred to SF seed in terms of consistency, texture and overall acceptability. The *Bacillus subtilis* BaAP3 strain could be effectively utilized for the fermentation of ALB seed for uniform product quality in the food condiment industry.

Keywords: *Bacillus subtilis*, fermentation, African locust bean seed, properties.

Introduction

African locust bean (ALB) (*Parkia biglobosa*) is a legume belonging to the sub-family Mimosoideae in the Leguminosae family. Processing of ALB fruits involves many different post-harvest operations such as pod and pulp removal to produce the seeds, cleaning, boiling, dehulling, washing, re-cooking, and then fermentation of the seeds to produce food condiment (Akande *et al.*, 2010). Fermentation involves the utilization of mixed or pure culture of microorganisms for bioprocessing or modification of a food substrate. The traditional fermentation of ALB seed is spontaneous and spearheaded by natural microflora with alkaline pH (Wang, 2017). Spontaneously-fermented ALB seed is high in protein (Achinewhu, 2015); minerals, especially calcium (122 mg/100g) and phosphorus (297 mg/100g) (Sung *et al.*, 2005) and poly- γ -glutamic

acid as part of the synthesized mucilaginous materials (Oyedokun, 2016).

Bacillus was first reported by Christian Gottfried Ehrenberg in 1835 (Sella *et al.*, 2015). Since then, *Bacillus* has been found in a wide variety of organisms, such as pigs, environments such as ponds and soil (Borsodi *et al.*, 2011; Gu *et al.*, 2015; Wei *et al.*, 2016). *Bacillus* species, which are Gram-positive spore formers (Cutting, 2011), are widely documented as primary agents in the alkaline fermentation of legumes (Tamang *et al.* 2016) including ALB. The microbial species involved vary and most times this leads to variations in product quality (Diawara *et al.*, 1992; Sanni, 1993). Campbell (2016) reported 83% to 93% of the total isolate in *iru* to be *Bacillus* species while other organisms constituted 7% to 17% of the isolates. Also, Falegan (2017) isolated a percentage of 19.4% *Bacillus*

species from *iru* samples obtained from different sources. Enujiugha (2009) specifically identified *Bacillus subtilis* as the predominant organism that is responsible for the fermentation of protein-rich oil seeds to give the desired fermented products including *iru*.

The *Bacillus subtilis* BaAP3 strain is known to synthesize the highest concentration of a low molecular weight poly-gamma-glutamic acid (PGA) on the third of fermentation and the biopolymer is found to be indispensable to gastrointestinal mineral (calcium) absorption (Oyedokun *et al.*, 2020). Food manufacturers are constantly exploring ways of ensuring uniform quality, optimizing product acceptance, and increasing process efficiency (Pomeranz and Meloan, 2002). Utilizing pure culture of *Bacillus subtilis* BaAP3 strain has the potential of serving this purpose during the fermentation of ALB seed. Also, studies on nutritional, colour and sensory properties of ALB fermented by *Bacillus subtilis* BaAP3 strain are lacking. Therefore, this study was aimed at utilizing pure culture of *Bacillus subtilis* BaAP3 strain for the fermentation of ALB seed and to assess the nutritional, colour and sensory properties of the fermented products for possible industrial application towards ensuring minimal variation in the quality of fermented ALB seeds.

Material and Methods

Collection and preparation of African locust bean seeds

Dried ALB seeds were sourced from Oja-Oba, Akure, Nigeria. The seeds were prepared as described by Aderibigbe *et al.* (2014) by soaking in water (ratio 1:5 w/v) for 2 h, autoclaved at 121 °C for 15 min to quicken the cooking process and dehulled manually followed by storage under freezing conditions for preservation until required for fermentation.

Fermentation of African locust bean seeds

The prepared ALB seeds were subjected to fermentation as described by Aderibigbe *et al.* (2014) and Oyedokun *et al.* (2020). Two portions of 300 g cooked cotyledons in aluminium containers with paper laminated aluminum foil lid were sterilized in autoclave at 121 °C for 15 min and allowed to cool to room temperature. One portion was made to undergo spontaneous fermentation (SF), while the other was inoculated with *Bacillus subtilis* BaAP3 culture of concentration 8.4×10^8 cells/ml and thoroughly mixed followed by *Bacillus subtilis* BaAP3 fermentation (BSF). The fermentation process was carried out at 37 °C for 3 days. Thereafter, the fermented samples were subjected to nutritional, colour and sensory analyses.

Proximate composition

The proximate composition was determined using AOAC (2019). The dried matter (DM) was calculated from the moisture content of wet samples using the following relation,

$$DM = 100\% - \% \text{ moisture.}$$

While the carbohydrate content was determined by difference method:

$$\% \text{ Carbohydrate} = 100 - (\% \text{ moisture} + \% \text{ crude fat} + \% \text{ ash} + \% \text{ crude fibre} + \% \text{ crude protein}).$$

The calorific value of the samples was estimated by multiplying the percentages of crude protein, crude lipid and carbohydrate with the recommended factors of 2.44, 8.37 and 3.57, respectively (Ihedioha and Okoye, 2011).

Mineral analysis

The mineral analysis was carried out as described by AOAC (2019) using Atomic Absorption Spectrophotometer model 210 VGP (Buck Scientific, USA) for all the minerals except phosphorus which was determined by using Ultraviolet-Visible Spectrophotometer (Shimadzu Corporation, Japan). All values were expressed in mg/kg. The mineral ratios: calcium (Ca)/phosphorus (P), sodium (Na)/potassium (K), calcium (Ca)/potassium, sodium (Na)/magnesium, zinc (Zn)/copper (Cu) and iron (Fe)/copper (Cu) were calculated and compared with acceptable ranges as described by David (2010).

Colour measurement

The colour of the samples was measured using Colour Meter PCE-CSM 2 (Deutschland, GmbH) in connection with CQCS3 software. The colour difference of the samples were read in L* (brightness – 0 : black, 100 : white), a* (+ : red, - : green), b* (+ : yellow, - : blue) values (Yangilar, 2016). While the c* and h* values were calculated from *a and *b (Hirata, 2020).

Sensory evaluation

Five grammes (5 g) fermented samples were assessed by thirty panelists who were familiar with fermented ALB seed by using the method described by Larmond (1977). The properties: consistency (i.e. viscosity of film on the surface of cotyledons), texture, colour, odour (level of detection of ammoniacal odour) and overall acceptability were scored on a 9-point Hedonic scale; where 9 and 1 were 'like extremely' and 'dislike extremely' respectively.

Statistical Analysis

The data were analyzed using IBM SPSS version 20.0. The

mean and standard error of means (SEM) of triplicate determinations were calculated.

Results and Discussion

The proximate composition of spontaneously-fermented (SF) and *Bacillus subtilis* BaAP3 fermented (BSF) ALB seeds is presented in Table 1. There was no significant difference ($p>0.05$) in the moisture, dry matter, and ash contents between the SF and BSF samples. The moisture content values of the fermented samples were higher than 33.9% and 39.50% reported for fermented *Parkia biglobosa* seed and Bombax, respectively (Osuntokun, 2020) and higher than 44.74% obtained for partially fermented *Parkia biglobosa* and 51.54% reported for completely fermented locust bean seed (Oluwaniyi and Bazambo, 2016). The observed high moisture content of the samples could be as a result of high cooking temperature and pressure. It could also be as a result of metabolic activities of microorganisms during fermentation period (Oluwaniyi and Bazambo, 2016). The ash contents obtained in this study were lower than 7.0 g/100g obtained for fermented African locust bean (Adeyeye *et al.*, 2002), 6.21% reported for processed locust bean and 6.9% obtained for mesquite bean (Aremu *et al.*, 2015). The low ash contents in the fermented samples may be due to leaching during processing.

The crude protein content increased while carbohydrate content reduced significantly ($p<0.05$) for both samples. The increased protein content in the samples could be as a result of fermentation. This agrees with the report given by Adeyeye *et al.* (2002) in which fermentation resulted in increase in crude protein of ALB seed from 24.1 g/100g to 41.3 g/100g. However, Oluwaniyi and Bazambo (2016) recorded reduction in protein content from 42.04% (for raw seed) to 15.09% (for partially fermented seed) and 13.75% (for completely fermented seed). ALB could therefore be used as an alternative source of protein in the diet or as a protein supplement especially in nation like Nigeria where

the majority of the population lives on starchy food (Aremu *et al.*, 2015).

The carbohydrate contents of both fermented samples were lower than 24.2% obtained for fermented *Parkia biglobosa* seed (Osuntokun, 2020) and 41.10 % obtained for dehulled and defatted African locust bean seed (Elemo *et al.*, 2011). The loss in carbohydrate during processing could be attributed to leaching of some soluble carbohydrates during prolonged cooking and hydrolysis of starch to fermentable sugars which are utilized as carbon sources by the fermentative microorganisms (Yagoub *et al.*, 2004; Azokpota *et al.*, 2006).

The crude fat contents of both fermented samples were comparatively higher than 18.64 g/100g for germinated African locust bean and 18.63 g/100g for fermented African locust bean as reported by Ijarotimi and Keshinro (2012). The crude fat content for SF sample increased while there was no significant increase ($p>0.05$) in BSF samples during fermentation. The high crude fat contents could be as a result of drying which reduced the moisture in the samples.

For crude fibre, there was no significant difference ($p>0.05$) in SF samples while there was significant increase ($p<0.05$) in the BSF samples during the fermentation period. The crude fibre of both samples were relatively low compared to the values obtained for fermented *Parkia biglobosa* (5.94%) (Osuntokun, 2020) and fermented African locust bean (6.65%) (Adeyeye *et al.*, 2002). The low crude fibre may have stemmed from degradation of fibrous materials by enzymes produced by the fermentative organisms.

However, the energy content of SF sample was significantly ($p<0.05$) lower than that of BSF sample. The energy values of fermented ALB seed samples are comparable to the one (457.20 kcal) reported for fermented African locust bean flour by Ijarotimi and Keshinro (2012). The high energy value of the samples may be as result of high crude fat content as fat contains more energy than any other macronutrients.

Table 1: Proximate composition (g/100g DM) and energy values (Kcal/100g DM) of spontaneous and *Bacillus subtilis* fermented African locust bean seeds

Component	SF ₀	SF ₃	BSF ₀	BSF ₃
Moisture	60.71±0.73a	63.13±0.66 ^a	63.53±0.62 ^a	63.12±3.44 ^a
Dry matter	39.29±0.73 ^a	36.87±0.66 ^a	35.87±0.50 ^a	36.41±3.70 ^a
Ash	1.30±0.57 ^a	1.29±0.55 ^a	0.97±0.01 ^a	0.97±0.01 ^a
Crude protein	44.36±0.00 ^a	53.91±8.08 ^b	35.50±0.10 ^a	49.70±0.10 ^b
Carbohydrate	27.24±0.45 ^a	0.77±0.43 ^b	23.48±1.19 ^a	10.88±1.46 ^b
Crude fat	25.57±0.56 ^b	38.22±0.42 ^a	38.24±1.06 ^a	37.54±1.47 ^a
Crude fibre	1.49±0.17 ^a	1.48±0.17 ^a	1.81±0.16 ^a	0.90±0.00 ^b
Energy/Kcal	418.56±4.64 ^a	465.58±2.11 ^b	487.88±2.31 ^b	474.36±7.08 ^c

Values are means of triplicate determinations (mean ± standard deviation)

Means in the same row with different superscript letter are significantly different (p < 0.05).

SF₀ = 0 day spontaneously fermented ALB seed; SF₃ = 3 day spontaneously fermented ALB seed;

BSF₀ = 0 day Bacillus subtilis BaAP3 fermented ALB seed; BSF₃ = 3 day Bacillus subtilis BaAP3 fermented ALB seed.

The mineral levels and ratios for the samples are shown in Table 2. The calcium content reduced from 1075.00 to 1010.33 mgkg⁻¹ for SF sample and increased from 1179 to 1221.33 mgkg⁻¹ for BSF sample. The sodium and copper contents of both SF and BSF samples reduced significantly (p<0.05) on fermentation. The sodium content reduced from 670.10 to 490.07 mgkg⁻¹ for SF sample and from 740.20 to 600 mgkg⁻¹ BSF sample, while copper content reduced from 5.00 to 3.00 mgkg⁻¹ for both samples. The magnesium, iron and zinc contents for SF and BSF samples were not significantly different. For potassium and phosphorus, the values increased for both samples.

The potassium contents increased from 571.67 to 719 mgkg⁻¹ and 580.00 to 631.67 mgkg⁻¹ for SF and BSF samples, respectively. While the phosphorus content increased from 2856.5 to 3244.4 mgkg⁻¹ for SF sample and 2810.80 to 2970.17 mgkg⁻¹ for BSF sample. The changes recorded in the mineral contents are in agreement with some previous findings that fermentation led to changes in mineral contents of African locust bean seed. Adeyeye *et al.* (2002) reported an increase in calcium content and decrease in such minerals as magnesium, sodium, potassium, phosphorus, zinc and iron for fermented African locust bean. Ijarotimi and Keshinro (2012) observed similar trend for African locust bean during its fermentation. However, fermented ALB seed is a good source of calcium, magnesium, phosphorus, zinc and iron. This means the fermented products could contribute to meeting the recommended daily allowance requirements of these minerals.

The mineral ratios are often important than their individual mineral levels (David, 2010). The Ca/P ratios for both samples were below ideal values (1.8-3.6) for good nervous activity (David, 2010). Ca/P ratios that are higher or lower than ideal negatively impact overall nervous system function (David, 2010). For Na/K, fermentation resulted in reduction for both SF and BSF samples. The values obtained were below the range (1.4-3.4) considered to be acceptable for an ideal adrenal activity (David, 2010). In the case of Ca/K, the ratios for both samples were below the ideal ratio (4) required by the body (David, 2010). However, the samples could contribute to calcium intake in diets. The Na/Mg ratio (2.27) for BSF sample was within the range (2-6) for normal adrenal activity (David, 2010). This suggests that if BSF could be consumed in sufficient quantity it may address underactive adrenal gland. The Ca/Mg for BSF (4.63) was higher than that of SF (4.04). These values are within the optimal limits (3-11) considered to be acceptable for optimal glucose metabolism as calcium is required for the release of insulin from the pancreas while magnesium inhibits insulin secretion (David, 2010). The Zn/Cu ratio for BSF sample (8.34) was comparable to the acceptable range (4-12) (David, 2010). While the Fe/Cu ratio for both samples were higher than the acceptable range (0.2-1.6) for normal cellular activity (David, 2010).

The colour properties of SF and BSF of ALB seeds are shown in Table 3. There was no significant difference (P > 0.05) in the values of the properties for both samples and the values suggested that the colour was grayish.

Table 2: Mineral composition (mgkg⁻¹) and mineral ratios of spontaneously fermented (SF) and *Bacillus subtilis* fermented (BSF) African locust bean seeds

Mineral	SF ₀	SF ₃	BSF ₀	BSF ₃
Composition (mgkg⁻¹)				
Calcium	1075.00±1.00 ^a	1010.33±0.58 ^b	1179.00±1.00 ^d	1221.33±1.53 ^c
Sodium	670.10±0.90 ^a	490.07±1.01 ^b	740.20±0.98 ^d	600.00±1.00 ^c
Copper	5.00±0.10 ^a	3.00±0.10 ^b	5.00±0.10 ^a	3.00±0.10 ^b
Magnesium	266.00±1.00 ^a	264.00±1.00 ^a	267.00±1.00 ^c	267.00±1.00 ^c
Iron	22.00±1.00 ^a	24.00±1.00 ^a	35.00±1.00 ^d	24.00±1.00 ^{ac}
Zinc	23.00±1.00 ^a	29.00±1.00 ^b	25.00±0.00 ^c	25.00±0.00 ^c
Potassium	571.67±1.53 ^a	719.00±7.81 ^b	580.00±1.00 ^d	631.67±1.53 ^c
Phosphorus	2856.50±0.10 ^b	3244.40±0.10 ^a	2810.80±1.00 ^c	2970.17±0.06 ^d
Mineral Ratios				
Ca/P	0.38 ±0.00 ^a	0.31±0.00 ^b	0.42±0.00 ^c	0.41±0.00 ^d
Na/K	1.17±0.00 ^a	0.68±0.01 ^b	1.28±0.00 ^c	0.95±0.00 ^d
Ca/K	1.88±0.00 ^a	1.41±0.02 ^b	2.03±0.00 ^c	1.93±0.00 ^d
Na/Mg	2.52±0.01 ^a	1.86±0.00 ^b	2.77±0.01 ^c	2.27±0.01 ^d
Ca/Mg	4.04±0.01 ^a	3.83±0.15 ^b	4.42±0.02 ^c	4.63±0.02 ^d
Zn/Cu	4.60±0.30 ^a	9.67±0.01 ^b	5.00±0.10 ^a	8.34±0.28 ^b
Fe/Cu	4.40±0.11 ^a	8.01±0.60 ^b	7.00±0.18 ^b	8.01±0.51 ^b

Values are means of triplicate determinations (mean ± standard deviation)

Means in the same row with different superscript letter are significantly different ($p < 0.05$)

SF₀ = 0 day spontaneously fermented ALB seed; SF₃ = 3 day spontaneously fermented ALB seed; BSF₀ = 0 day *Bacillus subtilis* BaAP3 fermented ALB seed; BSF₃ = 3 day *Bacillus subtilis* BaAP3 fermented ALB seed.

Table 3: The colour properties of spontaneously fermented (SF) and *Bacillus subtilis* BaAP3 fermented (BSF) African locust bean seeds

Sample	L*	a*	b*	c*	H*
SF					
SF ₀	33.98±3.10 ^a	10.03±0.34 ^a	16.86±3.28 ^a	19.66±2.86 ^a	58.81±4.76 ^a
SF ₃	31.67±3.44 ^a	9.29±0.58 ^a	17.36±2.01 ^b	17.36±2.01 ^a	61.74±1.49 ^a
BSF					
BSF ₀	33.16±6.86 ^a	10.11±2.80 ^a	17.20±6.89 ^a	19.98±7.31 ^a	58.69±3.81 ^a
BSF ₃	34.96±1.01 ^a	9.30±1.06 ^a	13.19±4.71 ^a	13.19±4.71 ^a	53.69±6.23 ^a

Values are means of triplicate determinations (mean ± standard deviation)

Means in the same row with different superscript letter are significantly different ($p < 0.05$)

SF₀ = 0 day spontaneously fermented ALB seed; SF₃ = 3 day spontaneously fermented ALB seed;

BSF₀ = 0 day *Bacillus subtilis* BaAP3 fermented ALB seed; BSF₃ = 3 day *Bacillus subtilis* BaAP3 fermented ALB seed.

The sensory properties of the fermented products (Table 4) indicate that BSF seed was preferred to SF seed in terms of consistency, texture and overall acceptability. This agrees to the findings of Enujiugha (2009) who reported that *Bacillus subtilis* fermented *iru* was preferred by consumers and that *Bacillus subtilis* was predominantly responsible for the fermentation of protein-rich oil seeds to give the desired fermented products (*ogiri*, *iru*, *ugba* and *okpei*). However, there was no significant difference ($p > 0.05$) in their colour and odour.

Table 4: Sensory properties of spontaneous fermented (SF₃) and *Bacillus subtilis* BaAP3 fermented (BSF₃) African locust bean seeds

Sensory property	SF ₃	BSF ₃
Consistency	5.57±1.79 ^b	6.77±1.04 ^a
Texture	5.77±1.65 ^b	6.77±1.25 ^a
Colour	5.87±1.81 ^a	6.63±1.27 ^a
Odour	5.80±1.73 ^a	6.10±1.65 ^a
Overall Acceptability	5.93±1.72 ^b	6.83±1.46 ^a

Values are means of triplicate determinations (mean ± standard deviation)

Means in the same row with different superscript letter are significantly different ($p < 0.05$)

SF₃ = 3 day spontaneously fermented ALB seed; BSF₃ = 3 day *Bacillus subtilis* BaAP3 ALB fermented seed.

Conclusion

Fermentation increased protein and crude fat contents with concurrent reduction in carbohydrate. The high calcium content of the fermented seed especially BSF sample, implies that the product could be utilized for preventing and ameliorating osteoporosis. Reduction in sodium on fermentation could make the product suitable for patient with hypertension. The colour of all the samples approached grey but BSF seed sample was preferred in terms of consistency, texture and overall acceptability to SF seed sample. The *Bacillus subtilis* BaAP3 strain could be effectively utilized for the fermentation of ALB seed for uniform product quality in the food condiment industry.

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