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Effects of starter culture on the Proximate, Antioxidant, Antinutritional and Mineral composition of fermented *Parkia biglobosa* seeds to produce Iru

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Abstract
Iru is a fermented condiment obtained from the seeds of *Parkia biglobosa*. This research was designed to investigate the effect of using different starter culture on the proximate, antioxidant, anti-nutritional and mineral composition of fermented *Parkia biglobosa* seeds. The raw seeds were dehulled and fermented naturally and by using starter culture of *Bacillus subtilis*, *Lactobacillus plantarum* and *Leuconostoc* sp. for 72h. The bacterial load of the samples fermented with *Bacillus subtilis* was not significantly different from the naturally fermented with a value of 8.65$\log_{10}$cfu/g. The *Parkia biglobosa* seeds fermented with *L. plantarum* had the highest titratable acidity (TTA) (0.113N) and the lowest pH (6.63) after 72 hours of fermentation. The highest protein was recorded in samples fermented with *Bacillus subtilis* with a value of 40.36%. Only sample fermented with *Leuconostoc* sp. showed the presence of copper at 0.23mg/g level after fermenting for 72hr. The anti-nutritional factor showed that trypsin inhibitor was 38.02 mg/g in the raw seeds and this reduced to 17.54mg/g after natural fermentation for 72h and was 26.46mg/g in samples fermented with *L. plantarum*. The least phytate (10.96mg/g) and oxalate (1.03mg/g) concentration were found in sample fermented with *B. subtilis*. 'Iru' fermented with *B. subtilis* displayed a higher antioxidants property except in 2, 2, azinobis-3-ethylbenzothiazoline-6-sulphuric acid radical (ABTS) where the 'iru' fermented with *Leuconostoc* sp was the highest with a value of 0.0204mg/g. The naturally fermented iru had the highest tannin with a value of 2.87mg/g, the highest saponin was found in iru fermented with *L. plantarum* with a value of 58.29mg/g. The highest glycosides and terpinoid were found in iru fermented with *Leuconostoc* sp. The organoleptic analysis showed that the ammonia odour was highest in naturally fermented 'iru' followed by that fermented by *B. subtilis*. The texture showed that 'iru' fermented with *L. plantarum* was much softer compared to others. The acceptability showed that 'iru' fermented with *B. subtilis* and naturally fermented was well accepted by consumers. On the basis of the acceptability as well as the nutritional composition, *Bacillus subtilis* can be recommended in the fermentation of *Parkia biglobosa* seeds to produce good quality iru.

Keywords: Iru, *Bacillus subtilis, Lactobacillus plantarum*, proximate

1. Introduction

Foods are often fermented to produce varieties of a better quality in terms of digestibility, nutrient and degrading hazardous components in the raw material [1]. The effect of fermentation can never be overemphasized, it has been reported that fermentation process degrades some hazardous chemicals and accumulate secondary metabolites. Fresh cassava contains chemicals like cyanide and are degraded during fermentation and rendered safe for consumption [1]. *Parkia biglobosa* is a legume that produces seeds that are fermented into iru. The fermented seeds of *Parkia biglobosa* are used in all part of Nigeria as soup condiment [2]. Iru is rich in protein and this often serves as a cheap source of protein to rural dwellers [3]. Based on the nutritional composition of this fermented condiment, it is imperative to improve on the fermentation procedure since the method that is conventionally used in the production of 'iru' is left to the microflora (chance inoculation) of the seeds. Locally, Iru is produced through natural/uncontrolled fermentation. This may lead to the accumulation of bioactive substance produced by other contaminating organisms on the final product. The present study is therefore meant to investigate the effects of starter culture on the proximate, mineral, antinutrient and antioxidant properties of *Parkia biglobosa* fermented to produce iru.

2. Method

2.1 The seeds of *Parkia biglobosa* used in this research was bought from Oja Oba, a local market in Omuo-Ekiti, Ekiti state, Nigeria.

2.2 Production of iru

Iru was produced using the modified Omafuvbe *et al.* [4] method. The flow chart below (Fig 1) shows the unit operations involved in the production of iru.

Parkia biglobosa seeds
↓
Soaked for 30 mins
↓
Cooked under pressure (2hr)
↓
Dehulled
↓
The cotyledons washed
↓
Re-cooked for (1h) under pressure
↓
Excess water drained
↓
Poured into sterile fermenters
↓
Starter culture introduced
↓
Incubated for 72h

Fig 1: Flow chart for the production of Iru

2.3 Microbial Analysis
The microbiological procedure was carried out following standard procedures. The starter cultures were activated on 1.3% buffer peptone water, the turbidity was matched to 0.5MaCfaland standard and diluted to give 10^4cell/ml [3]. The bacteria load was determined using a standard procedure as documented by Osho *et al*. [5]. The microbial analysis was carried out on the fermenting beans to confirm the presence of the starter culture only.

2.4 The physicochemical properties
The proximate, pH, titratable acidity, mineral, antioxidant and antinutritional factors were carried out using standard procedure of AOAC [6].

2.5 Determination of the sensory attributes of samples.
Thirty (30) panelist were used to organoleptically determine the differences in the intensity of bacterial film growth on the surface of the cotyledons, the change in texture, colour, and the difference in the level of ammonia odour, the overall liking of each sample was also assessed.

2.6 Statistical Analysis
All data collected were subjected to statistical analysis using statistical package for social sciences, means were compared using New Duncan's multiple range test (SPSS version 16.0 2010)

Results
The bacteria load of the fermented seeds was highest for the naturally fermented sample though there was no significant difference ($P \geq 0.05$) between the bacterial load of the naturally fermented and the sample fermented with *Bacillus subtilis*. *Lactobacillus* had the least load (6.15 $\log_{10}$cfu/g) as shown in Table 1. The titratable acidity (TTA) was highest in the sample fermented with *Lactobacillus plantarum* with a value of 0.133N. The TTA of the naturally fermented was lowest with 0.044N. The pH of samples fermented with *L. plantarum* was found to be 6.63 and the least in the series of fermented samples.
Figure 2 shows the proximate composition of the unfermented and the fermented seeds. The carbohydrate of the samples fermented with *Lactobacillus* was highest after 72 h of fermentation while the sample naturally fermented had the least. The protein content of sample fermented with *Bacillus subtilis* was found to be 40.36% which was highest. There was no significant difference ($P \geq 0.05$) in the crude fat of all the samples fermented with *Lactobacillus* sp, *Leuconostoc* sp, *Bacillus* sp and natural fermentation. The crude fibre of the samples fermented with *Lactobacillus* and *Leuconostoc* were not significantly different ($P \geq 0.05$) from the unfermented seeds. Table 2 shows the mineral composition of the fermented samples. Potassium, calcium, and phosphorus levels increased after the fermentation in all the fermented samples. Manganese was not detected in samples fermented with *Lactobacillus plantarum* and *Leuconostoc* sp. Table 3 shows the anti- nutritional factor in the fermented samples. Trypsin inhibitor was highest in the unfermented seeds with a value of 38.3mg/g and was least in samples fermented naturally with a value of 17.54mg/g, although, there was no significant different ($P \geq 0.05$) in the values found for *Leuconostoc* sp, *Bacillus subtilis* and naturally fermented. The phytate and oxalate content reduced in all the fermented samples.
The antioxidant composition is presented in Table 4. The highest flavonoid and phenol were recorded in samples fermented with *Bacillus subtilis*. Ferric reducing antioxidant power (FRAP) and 2, 2 diphenyl-1-picrylhydrazyl (DPPH) activity of the samples fermented with *Bacillus subtilis* was also high. 2, 2, azinobis-3-ethylbenzothiazoline-6-sulphuric acid radical (ABTS) was found to be highest in sample fermented with *Leuconostoc* sp. (Table 5).
Figure 3 shows the effect of different starter culture on the phytochemical composition of the samples. Tannin was found to be highest in naturally fermented sample while saponin was highest in sample fermented with *Lactobacillus plantarum*. Glycosides and terpinoids were found highest in samples fermented with *Leuconostoc* sp.
The organoleptic evaluation of the samples showed that the intensity of bacterial growth was highest on the samples naturally fermented, though not significantly different ($P \geq 0.05$) from the sample fermented with *Bacillus subtilis*. The sample fermented with *Lactobacillus plantarum*, *Bacillus subtilis* and naturally fermented were not significantly different ($P \geq 0.05$) in their texture. The colour of the cotyledon of the fermented seed became darker compared to the unfermented. The ammonia odour was highest in the naturally fermented sample. The overall-liking showed that there was no significant difference in the acceptability of sample fermented with *B. subtilis* and naturally fermented 'iru' (Figure 4).

Table 1: Bacterial load, pH, and Titratable acidity (TTA) of unfermented and fermented *Parkia biglobosa* seeds.

Samples	Bacterial load ($\log_{10}$cfu/g)	pH	Titratable acidity (N)
Unfermented	0.00±00^a	5.50±0.05^a	0.177±0.004^d
Fermented with *Lactobacillus plantarum*	6.15±0.12^b	6.63±0.03^b	0.113±0.001^c
Fermented with *Leuconostoc* sp	7.74±0.08^c	7.03±0.03^c	0.103±0.006^c
Fermented with *Bacillus subtilis*	8.65±0.07^d	7.73±0.06^d	0.069±0.005^b
Natural fermentation	8.67±0.16^d	8.00±0.05^e	0.044±0.001^a

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at $P \geq 0.05$.

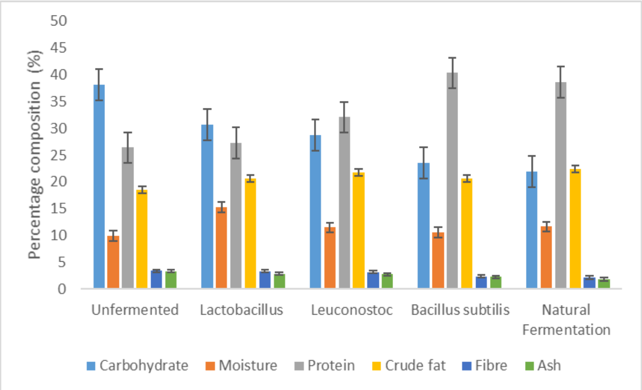

Fig 2: Proximate composition (%) of unfermented and fermented *Parkia biglobosa* seeds

Table 2: Mineral composition (mg/g) of unfermented and fermented *Parkia biglobosa* seeds.

Elements	unfermented	Lactobacillus plantarum	Leuconostoc sp	Bacillus subtilis	Natural Fermentation
Sodium	30.1±0.15^{ab}	29.40±0.1^{a}	30.50±0.2^{b}	33.3±0.5^{c}	33.23±0.8^{c}
Potassium	42.87±1.5^{a}	43.77±0.51^{a}	47.1±0.51^{b}	51.37±0.93^{c}	49.03±0.38^{bc}
Calcium	26.17±2.0^{a}	45.67±0.68^{b}	48.87±0.17^{c}	51.97±0.75^{c}	55.7±0.57^{d}
Magnesium	40.7±1.02^{a}	40.16±0.06^{a}	49.1±0.41^{d}	44.67±0.37^{b}	46.4±0.21^{c}
Zinc	2.26±0.08^{c}	2.56±0.07^{c}	1.39±0.02^{ab}	1.7±0.05^{b}	1.20±0.06^{a}
Iron	0.9±0.06^{a}	1.23±0.12^{b}	1.10±0.06^{ab}	1.53±0.08^{c}	1.50±0.05^{c}
Lead	-	-	-	-	-
Manganese	0.767±0.33^{b}	-	-	1.70±0.05^{d}	1.6±0.00^{c}
Cadmium	-	-	-	-	-
Cobalt	-	-	-	-	-
Nickel	-	-	-	-	-
Phosphorus	6.5±0.28^{a}	7.3±0.11^{a}	8.47±0.23^{b}	10.2±0.66^{d}	9.27±0.08^{cd}
Copper	0.467±0.03^{c}	-	0.23±0.06^{b}	-	

**Values in the row are the means of triplicate determinations and those with the same superscript are not significantly different, at P≥0.05.

Table 3: Anti-nutritional composition (mg/g) of unfermented and fermented *Parkia biglobosa* seeds

Samples	Trypsin inhibitor	Phytate	Oxalate
Unfermented	38.02±0.54^{c}	18.54±0.64^{d}	3.16±0.07^{d}
Fermented with Lactobacillus plantarum	26.48±1.0^{b}	13.25±0.07^{b}	2.58±0.08^{c}
Fermented with Leuconostoc sp	18.66±0.73^{a}	16.49±0.48^{c}	3.01±0.02^{d}
Fermented with Bacillus subtilis	18.84±0.45^{a}	10.96±0.28^{a}	1.03±0.02^{a}
Natural fermentation	17.54±0.53^{a}	11.98±0.39^{ab}	1.65±0.24^{b}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≥0.05.

Table 4: Anti-oxidant composition (mg/g) of unfermented and fermented *Parkia biglobosa* seeds.

Samples	Flavonoid	Phenol
Unfermented	0.172±0.00^{c}	10.86±0.03^{c}
Fermented with *Lactobacillus plantarum*	0.012±0.00^{a}	2.905±0.08^{a}
Fermented with *Leuconostoc* sp	0.087±0.00^{b}	4.48±0.08^{b}
Fermented with *Bacillus subtilis*	0.253±0.02^{d}	11.15±0.2^{d}
Natural fermentation	0.206±0.00^{c}	10.497±0.03^{c}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≥0.05.

Table 5: Anti-oxidant activities of unfermented and fermented *Parkia biglobosa* seeds.

Samples	ABTS (mMol/g)	FRAP (mg/g)	DPPH (%)
Unfermented	0.0149±0.0^{c}	0.548±0.2^{a}	24.43±0.05^{b}
Fermented with *Lactobacillus plantarum*	0.0125±0.0^{a}	1.293±0.43^{b}	14.43±0.25^{a}
Fermented with *Leuconostoc* sp	0.0214±0.0^{e}	6.660±0.1^{c}	27.27±0.7^{c}
Fermented with *Bacillus subtilis*	0.0186±0.0^{d}	7.608±0.07^{d}	69.04±0.51^{e}
Natural fermentation	0.0137±0.0^{b}	6.847±0.02^{c}	55.468±0.52^{d}

**Values along the column are the means of triplicate determinations and those with the same superscript are not significantly different, at P≥0.05.

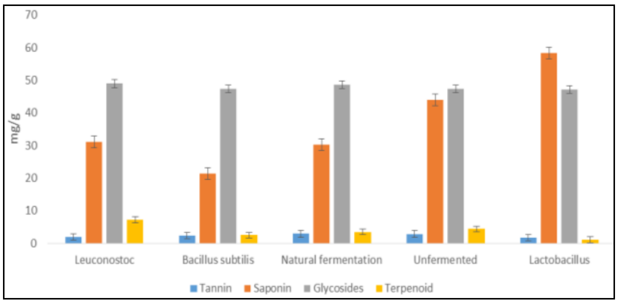

Fig 3: Phytochemicals present in *Parkia biglobosa* seeds fermented with different bacterial isolates

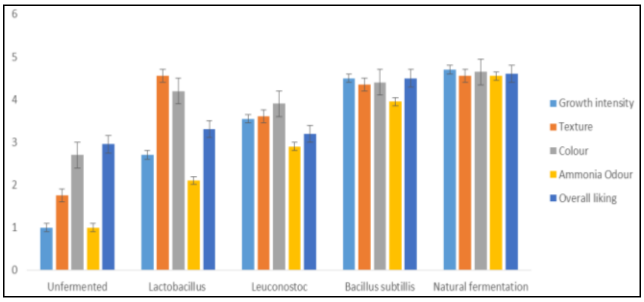

Fig 4: Sensory scores of fermented *Parkia biglobosa* seeds with different bacterial isolates.

Discussion

The bacterial load increased during fermentation. The increase in bacterial load may have resulted from the ability of the bacteria (*Lactobacillus*, *Leuconostoc* and *Bacillus*) to make use of the available nutrients in *Parkia biglobosa* in the production of more cells. A similar increase in bacterial load was previously reported by Adelekan and Nwadiuto [7] during the fermentation of *Parkia biglobosa* seeds to produce "iru". The highest bacterial load was found in samples naturally fermented, this may have been the result of some of the first colonizing bacteria producing secondary metabolite for the growth of others.

Titratable acidity (TTA) was highest in samples fermented with *Lactobacillus plantarum*, this may have been the result of the fact that *Lactobacillus* is a lactic acid fermenter. Moreover, it may have also been the resulting effect of the low pH observed in the samples fermented with *Lactobacillus plantarum*. The resulting increase in the pH and reduction in TTA of samples obtained by natural fermentation, *Bacillus subtilis* and *Leuconostoc* may have been the effect of accumulation of ammonia. A similar increase was also reported by Barber and Achinewhu [8] during the fermentation of *Citrullus vulgaris* to 'ogiri'. A decrease in TTA and increase in pH was also reported by Omafuvbe *et al*. [9] during the fermentation of *Prosopisa africanan*.

The increase in protein content of the fermented sample compared to the unfermented had earlier been reported [10]. Omafuvbe *et al*. [4] also reported a slight increase in the protein composition of fermented *Parkia biglobosa* seeds. Makonjuola and Ajayi [11] also observed a similar increase in protein content during fermentation of *Parkia biglobosa* seeds. The increase in the protein content was attributed to the proteolytic activities of the bacteria involved in the fermentation [12]. *Bacillus subtilis* fermented samples had the highest protein content which was similar to what Omodara and Aderibigbe [13] reported when starter culture of *Bacillus subtilis* was used during the fermentation of *Parkia biglobosa* seeds.

The reduction in the carbohydrate content may have been the effect of increase in population of the bacteria which resulted in the increase in metabolism thereby making use of the available sugar for energy and biomass production. Oluwaniyi and Bazambo [14] reported an increase in the crude fat and this increase was attributed to the loss of some other constituents of the seeds due to heat treatment during the fermentation process thereby leading to an overall increase in the fat content of the fermented seeds. The reduction in the ash content after fermentation was attributed to the loss of some mineral during the fermentation processes [14].

The accumulation of some element like calcium, potassium sodium and iron during fermentation of *Parkia biglobosa* seeds was earlier reported by Oluwaniyi and Bazambo [14]. Copper was not detected in the naturally fermented sample and sample fermented with *B. subtilis*, this may have resulted from the degradability potential of bacteria involved during the fermentation process.

The observed decrease in the trypsin inhibitor was also reported by El-Adawy [15] and Omodara and Aderibigbe [13]. The reduction was attributed to the action of bacteria during fermentation. The decrease in both Phytate and oxalate may be due to the activities of the enzymes produced by the fermenting bacteria [16]. A similar report on the reduction of phytate was also reported by Omodara and Aderibigbe [13].

Antioxidant compounds such as Phenol and flavonoid increased as the fermentation progressed, a similar report was given by Omodara and Aderibigbe [13] where sample fermented with *Bacillus subtills* had the highest phenol content and flavonoid. Arachaporn *et al*. [17] reported that the scavenging effect of DPPH in fermented sample of soy beans seeds depends on the starter culture, he observed that lactic acid bacteria (LAB) exhibited lower scavenging effect and Fe^{2+} chelating activity than sample fermented with *B. subtilis* which is similar to what is observed in this study. Oboh and Akindahunsi [18] reported that increase in the free radical scavenging level of fermented product is dependent on the ability of microbial enzymes to hydrolyze glycoside bonds thereby releasing free radical scavenging with more functional groups for antioxidant activities. The phytochemicals; Tannin, saponin, glycosides and terpinoids were still present in the fermented seeds; this indicated that the fermented seeds can still serve as a good source of phyto-nutrients.

The organoleptic evaluation indicated that the intensity of growth on the surface of the cotyledon is related to the bacterial load observed where both naturally fermented and *Bacillus* fermented samples had the highest as was observed in the sensory sampling. The fermentation had a softening effect on the *Parkia biglobosa* seeds. This observation is closely related to the report of Atere and Aderibigbe [19]. The samples became darker after fermentation, which is similar to the observation of Babalola and Giwa [20] who reported a change in the colour of soy beans during fermentation. This change was attributed to the degradation of protein, fat and nucleic acid [21]. The ammonia odour was highest in samples fermented naturally; this may have supported the claim of Aderibigbe *et al*. [3], who reported that starter culture delays the onset of the ammonia odour. There was no significant difference in the overall-liking of the naturally fermented sample and sample fermented with *Bacillus subtilis*, this actually support the earlier researchers who claim that *Bacillus subtilis* is the main fermenter of 'iru' [3, 22, 23].

Conclusion

There was a high yield of antioxidant and a low level of anti-nutritional factors in samples fermented with *Bacillus subtilis*, the protein content was found highest in samples fermented with *B. subtilis* and with a moderate ammonia odour, the overall liking of the sample fermented with *B. subtilis* compete favourably with no significant difference when compared with the naturally fermented, Based on these observations, *Bacillus subtilis* can be recommended as a better starter culture for the production of 'iru' with a better nutritional composition.

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