



Enhancing Nutritional Composition of Fermented *Parkia biglobosa* Seeds with Spices

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Introduction: Fermented *Parkia biglobosa* seed is an essential condiment in Nigeria for its unique flavor and nutritional value.

Aims: The study investigated the influence of spices such as *Aframomum melegueta* (alligator pepper), *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Myristica fragrans* (nutmeg), *Curcuma longa* (turmeric), *Xylopi aethiopica* (grains of Selim), *Chrysobalanus icaco* (cocoplum) and *Parinari excelsa* (skinplum) on the nutritional composition of the condiment.

Methodology: Thirty grams (30g) of dried spice was added to 300g of previously pressure cooked and dehulled locust beans in separate containers. Each was inoculated with *Bacillus subtilis* and fermented at 37°C for 36 hours.

Results: The phytic acid in alligator fermented 'iru', ginger fermented 'iru' and nutmeg fermented 'iru' were 4.53mg/g, 18.54mg/g and 20.19mg/g respectively, which were significantly lower than the phytic acid of the commercially produced 'iru' (21.40mg/g). Starter culture fermented 'iru', turmeric

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fermented 'iru' and cocoplum fermented 'iru' had significantly lower levels of trypsin inhibitors of 24.68mg/g, 26.13mg/g and 26.13mg/g, respectively when compared to commercially fermented 'iru'. Skinplum fermented 'iru' and cocoplum fermented 'iru' had significantly higher flavonoids levels. Alligator fermented 'iru' had significantly higher Vit. A (4.65), Vit. B1 (2.94), Vit. B2 (0.31mg/g) and Vit. B5 (0.08 mg/g) compared to commercially produced 'iru'. The protein digestibility of Nutmeg fermented 'iru' was significantly higher than commercially fermented 'iru'. However, other fermented samples had significantly reduced protein digestibility compared to commercially produced 'iru'.

Conclusion: To fortify and enhance nutritional composition of fermented *Parkia biglobosa* seeds, alligator pepper, skinplum and nutmeg may be used.

Keywords: Anti-nutrients; antioxidants; fermentation; in-vitro protein digestibility; iru; *Parkia biglobosa*; spices; vitamins.

1. INTRODUCTION

Parkia biglobosa is a tree of great importance as a source of edible products rich in protein, fat and carbohydrate. It is also a source of income for most rural households in the Western part of Nigeria [1]. *Parkia biglobosa* pulp is a good source of energy and vitamin C, while the fermented grains supply calcium, lipids and proteins to the diets of vulnerable populations in West Africa [2]. Although the amount of 'iru' (fermented seeds) eaten at every meal is relatively small, the fact that it is eaten regularly makes it an essential source of nutrients [3].

Different spices and herbs have been added to various food products for centuries, to contribute to the characteristic flavor and color of the final product [4]. Some spices such as garlic, nutmeg, ginger, paprika, rosemary, and sage possess potent antioxidants that can prolong the shelf life of some fermented foods. This is mainly because oxidation of lipids in food leads to the onset of off flavors, which could make the food product unacceptable for human consumption. A handful of spices have been also been reported to possess numerous health benefits. Adequate evidence exists to prove that spices and herbs possess anti-inflammatory, antitumorogenic, antioxidant, anticarcinogenic, and glucose- and cholesterol-lowering activities [5].

Fermentation is the chemical breakdown of complex organic substance into a simpler one by the action of microorganisms such as bacteria, fungi and yeast. At the local level, fermentation is achieved by the indigenous microflora or the addition of fermented materials from previous fermentation, known as backslopping [6]. The application of the modern biotechnology, like starter culture has been found to reduce fermentation time and guarantee product quality

[6]. A previous work discovered that using starter culture significantly improved the fermented products' approximate composition, mineral, vitamin content, and antioxidants and significantly reduced the anti-nutritional factors [7]. Considering the increasing demand for this fermented product as a substitute for protein, vitamins, minerals, and antioxidants, further improving its nutritional quality is needed by incorporating some spices during the second boiling before fermentation. Therefore, this research aims to enhance the nutritional composition of fermented *Parkia biglobosa* seeds with spices.

2. MATERIALS AND METHODS

2.1 Source of Materials

The African locust bean (*Parkia biglobosa*) seed used for the research was purchased from Central market, Kota-Ekiti, Ekiti State. The spices used were purchased from Oja-Oba in Ado-Ekiti, Ekiti State. Pure culture of *B. subtilis* (strain 3A) was obtained from the stock cultures kept in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State. This strain had been previously used by Omodara and Aderibigbe, [6] to produce 'iru-woro' (the firm-type of fermented *Parkia bilobosa* seeds).

Preparation of starter culture: The starter culture was prepared using the method of Omodara and Aderibigbe, [6].

Preparation of the spices: A hundred grams (100g) of each of the underlisted spices were weighed and processed. Roots of *Zingiber officinale* were washed and peeled to remove the outer layer and cut into smaller pieces. Likewise, the rhizome of *Curcuma longa* was washed, peeled and cut into smaller pieces. Seeds of *Myristica fragrans* were washed and grated using

hand grater. The seeds of *Perinari excelsa* were shelled. The seeds of *Chrysobalanus icaco*, *Xylopia aethiopica* and *Aframomum melegueta* were removed from the pod. *Allium sativum* bulbs were washed followed by the removal of the membranous skin, and cutting of the cloves into smaller pieces. All the samples were dried at 50°C using an oven until a constant weight was archived and finely ground using blender. 30g of the blended spices were used for the research.

2.2 Laboratory Production of 'iru'

The method of Omodara and Aderibigbe [6] was adopted. The seeds were soaked in water for 15 minutes, boiled under a pressure (by using pressure pot for two hours) and dehulled by rubbing between palms to remove the testa. Three hundred grams (300 g) of each cotyledon were weighed into nine different 1L beakers. The 30g cotyledons in the first beaker were used for analysis without fermentation (UnFI), 30g sample in the second beaker was poured into pressure pot, boiled for 1h, drained and aseptically poured into a sterile rectangular-shaped aluminum fermenting can (10cm x 20cm x 10cm) and was labeled as naturally fermented 'iru' (NaFI). Another 30g of the boiled cotyledons were poured into a fermenting can, allowed to cool, inoculated with the *Bacillus subtilis* using strain 3A and fermented for 35°C for 36 h (BuFI). Thirty grams (30g) each of finely ground spices were added separately to cotyledons in beakers 4, 5, 6, 7, 8, 9, 10, and 11. These were poured into separate pressure pots and boiled at 121°C for 1 h. The boiled cotyledons were poured aseptically into different sterile fermenting cans of the same dimension used above and they were labeled as AIFI ('iru' fortified with Alligator pepper), GiFI ('iru' fortified with Ginger), NuFI ('iru' fortified with Nutmeg), GaFI ('iru' fortified with Galic), TuFI ('iru' fortified with Tumeric), GsFI ('iru' fortified with Grain of selime), CoFI ('iru' fortified with cocoplum) and SkFI ('iru' fortified with Skinplum). All the eight samples were inoculated with 1.0ml of the starter culture *B. subtilis* 3A and were fermented at 35°C for 36h. The commercially fermented 'iru' (CmFI) was purchased from the market. The NaFI, BuFI and StFI served as controls.

2.3 Determination of Antinutritional Factors

2.3.1 Phytic acid

Wheelere and Ferrel [8] method determined phytic acid. Four grams (4 g) of finely ground

sample was soaked in 1 L of 2% HCl inside a conical flask for 3h and was filtered. Five milliliters (5 ml) of 0.03% NH₄SCN was added as indicator and 50 ml of distilled water also added. This was titrated against ferric chloride solution containing 0.05 mg of iron (Fe) per ml of FeCl₃. The iron equivalent was obtained and the phytate content in mg/100 mg of dried sample was calculated.

2.3.2 Trypsin inhibitor

The trypsin inhibitor activity (TIA) in the sample was determined according to the method of Smith et al. [9]. The digest contained 1.0 g of the sample, 40µg of trypsin and 2mg of N-alpha-benzoyl-DL-Arginine-Pnitroanilidehydrochloride. The absorbance was read at 410nm.

2.4 Determination of Antioxidants

2.4.1 Total phenol, flavonoids and free radical scavenger

The total phenol contents of the samples were determined using the method reported by Singleton et al., [10], while flavonoids content and 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical-scavenging ability of the samples were determined by the method of Meda et al. [11] and Gyamfi et al. [12], respectively.

2.5 Determination of Vitamins

Accurately weighed 5-10grams of finely ground fermented locust bean sample was transferred into a labelled glass vial. About 20-30mL of a suitable solvent such as methanol or acetonitrile was added to the vial, after which it was tightly capped and shaken vigorously for 15 minutes for complete dissolution. The sample was centrifuged to separate solid particles from the liquid extract. Clear supernatant was transferred into a clean glass vial for HPLC analysis [13].

2.6 Determination of Multi-Enzyme *In vitro* Protein Digestibility

A 200mg sample was weighed into 100ml flask containing 35ml sodium citrate buffer (0.1mol/L, pH 3.6) with pepsin (1.5g pepsin/L) as described by Ojokoh et al [14], with a slight modification. The resulting solution was incubated for 2 hours at 37°C for 15 minutes. The supernatant was decanted and the residue was collected, washed and spun for supernatant collection to assay for protein using BSA as the standard [15]. The result was expressed as the percentage of total protein digestibility.

3. RESULTS

Table 1 showed the anti-nutritional factors of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds. The level of phytic acid in AIFI, GiFI and NuFI was 4.53, 18.54 and 20.19 respectively, significantly lower than the levels of phytic acid in the CmFI (21.40). However, TuFI, GsFI, SkFI, CoFI had significantly higher levels of phytic acid than the commercially prepared iru. The trypsin inhibitor levels in UnFI, GaFI, and SkFI were 33.70, 33.69, and 33.57, respectively, significantly higher than that of CmFI (32.19). Samples BuFI, TuFI and CoFI had significantly lower level of trypsin inhibitors of 24.68, 26.13

and 26.13, respectively when compared with CmFI.

Table 2 showed the antioxidant levels of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds. Fermentation led to a significant increase in the antioxidant level of all the fermented products. SkFI (1.68) and CoFI had significantly higher flavonoids than the CmFI (1.26), while AIFI (0.94) and TuFI (0.84) had the least flavonoid contents. BuFI and CoFI had a significant higher phenol and DPPH contents than the CmFI while GiFI and AIFI had lower concentration of the antioxidant.

Table 1. Anti-nutritional compositions of unfermented ‘iru’ and spices fortified fermented ‘iru’

Samples	Phytate mg/g	Trypsin inhibitor
UnFI	53.56 ^a ±0.02	33.70 ^a ±1.20
NaFI	25.96 ^{bc} ±0.03	29.40 ^b ±0.08
BuFI	28.84 ^{bc} ±0.00	24.68 ^e ±0.12
AIFI	4.53 ^e ±0.58	28.86 ^c ±0.88
GiFI	18.54 ^d ±0.58	30.05 ^b ±0.49
NuFI	20.19 ^{cd} ±0.00	29.59 ^b ±0.08
GaFI	21.42 ^c ±1.17	33.69 ^a ±0.54
TuFI	53.56 ^a ±1.17	26.71 ^{cd} ±0.21
GsFI	36.26 ^b ±1.07	31.19 ^{ab} ±0.12
CoFI	25.96 ^{bc} ±1.53	26.13 ^d ±0.12s
SkFI	32.55 ^a ±0.00	33.57 ^a ±1.07
CmFI	21.40 ^c ±0.04	31.19 ^{ab} ±0.50

Legend: UnFI = Unfermented Iru, NaFI = Naturally Fermented Iru, StFI = Starter Culture Fermented Iru, AIFI =Alligator Fermented Iru, GiFI = Ginger Fermented Iru, NuFI = Nutmeg Fermented Iru, GaFI = Galic Fermented Iru, TuFI = Tumeric Fermented Iru, GsFI = Grain of Selime Fermented Iru, CoFI = Cocoplum Fermented Iru, SkFI = Skinplum Fermented Iru, CmFI = Commercially Fermented Iru

Table 2. Antioxidant level of unfermented ‘iru’ and spices fortified fermented ‘iru’

Samples	Flavonoids	Phenols	DPPH
UnFI	0.61 ^h ±0.02	60.02 ^g ±1.20	50.09±0.12
NaFI	1.01 ^e ±0.03	70.00 ^f ±0.08	71.90 ^e ±0.11
BuFI	0.84 ^g ±0.04	100.77 ^a ±1.07	76.46 ^g ±0.11
AIFI	0.94 ^f ±0.03	75.03 ^e ±0.85	56.72 ^f ±0.42
GiFI	1.01 ^e ±0.01	84.69 ^d ±1.07	66.83 ^e ±0.16
NuFI	1.19 ^d ±0.03	95.71 ^b ±0.85	71.92 ^c ±0.58
GaFI	1.21 ^{cd} ±0.03	83.64 ^d ±1.07	56.72 ^f ±1.51
TuFI	1.23 ^{cd} ±0.03	88.62 ^c ±0.86	69.26 ^d ±0.37
GsFI	1.26 ^c ±0.02	87.26 ^c ±0.21	68.63 ^d ±0.42
CoFI	1.43 ^b ±0.01	96.92 ^b ±0.50	73.51 ^b ±0.11
SkFI	1.68 ^a ±0.04	94.96 ^b ±1.07	71.37 ^c ±0.31
CmFI	1.26 ^c ±0.04	87.62 ^c ±0.50	73.50 ^b ±0.10

Legend: UnFI = Unfermented Iru, NaFI = Naturally Fermented Iru, StFI = Starter Culture Fermented Iru, AIFI =Alligator Fermented Iru, GiFI = Ginger Fermented Iru, NuFI = Nutmeg Fermented Iru, GaFI = Galic Fermented Iru, TuFI = Tumeric Fermented Iru, GsFI = Grain of Selime Fermented Iru, CoFI = Cocoplum Fermented Iru, SkFI = Skinplum Fermented Iru, CmFI = Commercially Fermented Iru

The vitamins of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds are presented in Table 3. There were variations in the vitamin contents of the fermented products; however, fermentation led to significant increase in the vitamin composition (except vitamin D) of all the fermented samples. AiFI had significantly higher vit. A (4.65), vit. B1 (2.94), vit. B2 (0.31) and vit. B5 (0.08) than the commercially produced iru.

The protein digestibility of the unfermented, starter culture fermented and spices fortified fermented *Parkia biglobosa* seeds are presented in Table 4. Fermentation also led to significant increase in protein digestibility of the fermented products. The protein digestibility of NuFI (12.45) was significantly higher than CmFI (12.06). However, other fermented samples had

significantly reduced protein digestibility compared to CmFI.

4. DISCUSSION

Though there was a reduction in phytic acid during fermentation, the relatively higher level of phytic acid in products fermented using turmeric, grain of Selim, skinplum and cocoplum when compared to starter culture fermented product and commercially produced iru could have been due to low phytase activities during fermentation. The addition of spices might have hindered the phytase activities [16]. The reduction of phytic acid in samples fortified with alligator pepper might be due to increase in phytase activity during fermentation.

Table 3. Vitamins of unfermented ‘iru’ and spices fortified fermented ‘iru’

Samples	A	B 1	B 2	B 3	B 5	B 6	B 7	B 9	B12	C	D	E	K
UnFI	3.35 ^{ef}	2.10 ^{bcd}	0.14 ^f	3.37 ^f	0.04 ^{cde}	0.12 ^d	5.04 ^f	3.13 ^b	2.63 ^d	7.89 ⁱ	21.0 ^a	23.12 ^g	12.05 ^c
NaFI	3.32 ^e	2.70 ^b	0.31 ^b	3.78 ^d	0.06 ^{abc}	0.10 ^d	5.04 ^f	3.11 ^{bc}	2.69 ^d	12.41 ^d	18.92 ^d	24.36 ^e	11.03 ^d
BuFI	3.35 ^{ef}	2.10 ^{bcd}	0.14 ^f	3.37 ^f	0.04 ^{cde}	0.12 ^d	5.04 ^f	3.13 ^b	2.63 ^d	7.89 ⁱ	21.01 ^a	23.12 ^g	12.05 ^c
AIFI	4.65 ^a	2.94 ^a	0.45 ^a	3.78 ^d	0.08 ^a	0.21 ^c	6.01 ^c	3.03 ^d	3.0 ^b	12.45 ^d	19.84 ^c	26.05 ^c	10.17 ^f
GiFI	3.71 ^{de}	2.12 ^{bcd}	0.31 ^b	3.55 ^e	0.05 ^{bcd}	0.23 ^c	6.07 ^c	3.11 ^{bc}	2.25 ^e	9.66 ^f	20.02 ^b	27.33 ^b	11.03 ^d
NuFI	3.50 ^{def}	2.45 ^{abc}	0.11 ^f	4.01 ^c	0.03 ^e	0.10 ^d	6.25 ^b	2.94 ^e	3.02 ^b	10.04 ^e	18.89 ^d	24.36 ^e	14.02 ^a
GaFI	3.89 ^{cde}	2.70 ^{ab}	0.21 ^e	3.96 ^c	0.05 ^{bcd}	0.28 ^{ab}	6.00 ^c	2.90 ^f	2.88 ^c	13.19 ^c	20.02 ^b	27.62 ^a	10.24 ^e
TuFI	3.35 ^{ef}	2.40 ^{abc}	0.32 ^d	3.72 ^f	0.06 ^{abc}	0.29 ^{ab}	6.23 ^b	3.01 ^d	3.00 ^b	17.38 ^a	18.92 ^d	22.89 ^h	13.06 ^b
GsFI	3.96 ^{bcd}	2.02 ^{cd}	0.20 ^e	4.01 ^c	0.07 ^{ab}	0.32 ^{ab}	5.39 ^e	2.68 ^g	2.83 ^c	8.12 ^h	20.04 ^b	25.04 ^g	11.04 ^d
CoFI	4.34 ^{abc}	1.77 ^d	0.31 ^b	4.22 ^a	0.05 ^{bcd}	0.23 ^e	6.43 ^a	3.08 ^c	2.69 ^d	13.46 ^b	19.75 ^c	23.44 ^f	12.09 ^c
SkFI	4.46 ^{ab}	2.00 ^{cd}	0.13 ^f	4.07 ^b	0.04 ^{de}	0.32 ^a	5.84 ^d	3.23 ^a	3.23 ^a	9.35 ^g	20.10 ^b	22.07 ⁱ	13.06 ^b
CmFI	3.05 ^f	2.12 ^{bcd}	0.14 ^f	3.96 ^c	0.03 ^e	0.21 ^c	5.04 ^f	2.63 ^d	3.0 ^b	8.12 ^h	18.92 ^d	24.36 ^e	11.03 ^d

Legend: UnFI = Unfermented Iru, NaFI = Naturally Fermented Iru, StFI = Starter Culture Fermented Iru, AIFI =Alligator Fermented Iru, GiFI = Ginger Fermented Iru, NuFI = Nutmeg Fermented Iru, GaFI = Galic Fermented Iru, TuFI = Tumeric Fermented Iru, GsFI = Grain of Selime Fermented Iru, CoFI = Cocoplum Fermented Iru, SkFI = Skinplum Fermented Iru, CmFI = Commercially Fermented Iru

Table 4. Protein digestibility of unfermented ‘iru’ and spices fortified fermented ‘iru’

Samples	Protein digestibility
UnFI	7.02 ^g ±0.11
NaFI	11.05 ^c ±0.05
BuFI	12.08 ^b ±0.08
AIFI	9.31 ^f ±0.11
GiFI	11.07 ^c ±0.17
NuFI	12.45 ^a ±0.11
GaFI	10.08 ^e ±0.02
TuFI	9.27 ^f ±0.13
GsFI	9.46 ^f ±0.15
CoFI	10.57 ^d ±0.13
SkFI	10.26 ^e ±0.23
CmFI	12.06 ^b ±0.13

Legend: UnFI = Unfermented Iru, NaFI = Naturally Fermented Iru, StFI = Starter Culture Fermented Iru, AIFI =Alligator Fermented Iru, GiFI = Ginger Fermented Iru, NuFI = Nutmeg Fermented Iru, GaFI = Galic Fermented Iru, TuFI = Tumeric Fermented Iru, GsFI = Grain of Selime Fermented Iru, CoFI = Cocoplum Fermented Iru, SkFI = Skinplum Fermented Iru, CmFI = Commercially Fermented Iru

Fermentation also reduced the level of trypsin inhibitors during fermentation which might be due to the enzymatic activities of the fermenting organisms. Compared to commercially produced iru, the reduction was most significant in the starter culture fermented product and iru fortified with turmeric and cocoplum. It might be because these spices enhanced the growth of the fermenting organism and hence have been able to break the anti-nutritional factor during metabolic activities of the fermenting microorganisms.

The addition of skinplum and cocoplum as spices in production of fermented *Parkia biglobosa* might have led to significant increase in the levels of total flavonoids. In contrast, the addition of cocoplum led to an increase in total phenol. This might be because these spices have enhanced the enzymatic activities of the fermenting organisms during fermentation. Only the starter culture fermented product had a higher level of DPPH while all the fortified fermented *Parkia biglobosa* had a lower level of DPPH. This reduction might have been due to formation of complexes with the anti-oxidants, thereby rendering them unavailable [17], [6]. The reduction might also be because the addition of spices reduced the ability of the fermenting organisms to secrete the enzymes responsible for the breakdown of the glycosidic bonds of these antioxidants. This result agrees with earlier reports on the total phenol content of many plant foods, which is proportional to the antioxidant capacity of the plant food [17].

The higher value of Vit. A, Vit. B1, Vit. B2, and Vit. B5 in the sample fortified with Alligator pepper could be due to the vitamins in the spice added during processing. The significant increase in Vit. B1(thiamine) and Vit. B2 (riboflavin) recorded when alligator pepper was used may be that the spice enhanced the activities of riboflavin synthase of *Bacillus subtilis* which is the major fermenting organism [18]. Other spices fortified fermented *Parkia biglobosa* seeds which produced lower levels of vitamins when compared to commercially produced iru could have been that these spices formed complexes the vitamins.

The highest level of protein digestibility in starter culture fermented iru could be attributed to the proteolytic activity of the fermenting organisms which has contributed to the increase in the in-vitro protein digestibility of the fermented products [18]. Olasupo and Okorie also reported

that microorganisms produce proteolytic enzymes during fermentation, which degrade proteins to readily digestible forms [18]. However, the reduction in protein digestibility when other spices were added could be due to the inhibitory activities of the spices on proteolytic enzymes during fermentation by the fermenting microorganisms [19].

5. CONCLUSION

This study revealed that only alligator pepper was able to reduce the anti-nutritional factor of the fermented *Parkia biglobosa* seeds while the skinplum was able to increase the level of the antioxidants when compared to the commercially produced iru. Alligator pepper as a spice in the fortification of fermented *Parkia biglobosa* seeds also led to increase in some of the vitamins while nutmeg was found to increase the protein digestibility of the fermented products when also compared with the commercially produce iru. Hence, in fortification of fermented *Parkia biglobosa* seeds to enhance its nutritional composition, alligator pepper, skinplum and nutmeg may be used.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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