

## Original Research Article

### ENHANCING NUTRITIONAL COMPOSITION OF FERMENTED *Parkia biglobosa* SEEDS WITH SPICES

#### ABSTRACT

**Introduction:** Fermented *Parkia biglobosa* (African locust bean) seed is an important condiment in Nigeria for its unique flavour and nutritional value. The study was carried out to investigate the influence of spices such as *Aframomum melegueta* (alligator pepper), *Zingiber officinale* (ginger), *Allium sativum* (garlic), *Myristica fragrans* (nutmeg), *Curcuma longa* (turmeric), *Xylocarpus aethiopicus* (grains of Selim), *Chrysobalanus icaco* (cocoplum) and *Parinari excelsa* (skinplum) on the nutritional composition of the condiment.

**Aims:** The study was carried out to investigate the influence of different spices fermented with locust beans on the anti-nutritional factors, antioxidants level, vitamins and protein digestibility of the condiment.

**Methodology:** One hundred grams of each of the spice was peeled, washed and dried till a constant weight was achieved. Thirty grams (30g) of each spice was added to 300g of previously pressure cooked and dehulled locust beans in separate containers. Each was sterilized and inoculated with *Bacillus subtilis*. All the samples were fermented at 37°C for 36h. All samples were assayed for antinutrients such as phytic acid and trypsin inhibitor; antioxidants such as total phenol, flavonoids and free radical scavengers; vitamins as well as *in vitro* protein digestibility using standard methods.

**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 fermented 'iru' and cocoplum fermented 'iru' had significantly lower level of trypsin inhibitors of 24.68mg/g, 26.13mg/g and 26.13mg/g, respectively when compared to commercially fermented 'iru'. Skinplum fermented 'iru' (1.68 mg/g) and cocoplum fermented 'iru' had significantly higher flavonoids than the commercially fermented 'iru'. Grain of selim fermented 'iru' (1.26 mg/g), while alligator fermented 'iru' (0.94 mg/g) and turmeric fermented 'iru' (0.84 mg/g) had the least flavonoids contents. 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' (12.45) was significantly higher than commercially fermented 'iru' (12.06). However, other fermented samples had significantly reduced protein digestibility when compared to commercially produced 'iru'.

**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 increased the vitamin contents while nutmeg increased the protein digestibility of the fermented products. To fortify fermented *Parkia biglobosa* seeds in order to enhance its nutritional composition, alligator pepper, skinplum and nutmeg may be used.

**Key words:** Iru, Anti-nutrients, Antioxidants, Vitamins, In-vitro, protein digestibility, *Parkia biglobosa*, Fermentation, Spices

## INTRODUCTION

In Nigeira, *Parkia biglobosa* is a tree of great importance as a source of edible products that is rich in protein, fat and carbohydrate. It is also a source of income for the vast majority of rural households in 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 rather small, the fact that it is eaten regularly makes it an important source of nutrients [3].

Different spices and herbs have been added to various food products for centuries, basically in order to contribute to the characteristic flavor and colour of the final product [4]. Some spices such as garlic, nutmeg, ginger, paprika, rosemary, and sage possess strong antioxidants that can prolong the shelf life of some fermented foods. This is mainly due to the fact that oxidation of lipids in food leads to the onset of off flavors, which could make the food product to become unacceptable for human consumption. A handful of spices have been also reported to possess numerous health benefits. Adequate evidence exists to prove that spices and herbs possess anti-inflammatory, antitumorigenic, 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 local level, fermentation is achieved by the indigenous microflora or addition of fermented materials from previous fermentation, the process known as backslopping[6]. Application of the modern biotechnology, like the use of starter culture has been found to reduce fermentation time as well as guarantee product quality [6]. From the previous work, it was discovered that, the use of starter culture improved greatly the proximate composition, mineral, vitamin content, antioxidants of the fermented products and a great reduction in the anti-nutritional factors [7]. Taking into consideration, the increasing demand on this fermented product as a substitute for protein, vitamin, mineral and antioxidants, there is need to improve further the its nutritional quality by incorporating some spices during second boiling before fermentation. The aim of this research is therefore to enhance nutritional composition of fermented *Parkia biglobosa* seeds with spices.

## MATERIALS AND METHOD

### 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 [6] to produce 'iru-woro' (the hard-type of fermented *Parkia bilobosa* seeds).

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

**Preparation of the spices:** Hundred grams (100g) of each of the underlisted spices was 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 *Perinariexcelsa* were shelled. The seeds of *Chrysobalanus icaco*, *Xylopi 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.

## Laboratory production of 'iru'

The method of Omodara and Aderibigbe [6] was adopted. The seeds were soaked in water for 15 min, boiled under pressure (by using pressure pot for 2 h), dehulled by rubbing between palms to remove the testa. Three hundred grams (300 g) each of the cotyledons were weighed into nine different 1L-beakers. The 30g cotyledons in first beaker was 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 × 20cm × 10cm) and was labeled as naturally fermented 'iru' (NaFI). Another 30g of the boiled cotyledons was poured into 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.

## Determination of Antinutritional Factors

### Phytic acid

The method of Wheelere and Ferrel [8] was employed in the determination of phytic acid. Four grams (4 g) of finely ground sample was soaked in 1 L of 2% HCl inside conical flask for 3h and was filtered. Five milliliters (5 ml) of 0.03% NH<sub>4</sub>SCN was added as indicator and 50 ml of distilled water also added. This was titrated against ferric chloride solution which contained 0.05 mg of iron (Fe) per ml of FeCl<sub>3</sub>. The iron equivalent was obtained and the phytate content in mg/100 mg of dried sample was calculated.

### 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.

## Determination of Antioxidants

### Total phenol, flavonoids and free radical scavenger

The total phenol contents of the samples were determined using the method reported by [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.

## 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].

## Determination of multi-enzyme in vitro Protein Digestibility

A 200mg of the 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 [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 as the percentage of total protein digestibility.

## RESULTS

Table 1 shows 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, which were significantly lower than the level of phytic acid in the CmFI (21.40). However, TuFI, GsFI, SkFI, CoFI had significantly higher level of phytic acid than the commercially prepared iru. The level of trypsin inhibitor in UnFI, GaFI and SkFI were 33.70, 33.69 and 33.57 respectively, which were 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 shows the anti-oxidant 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 flavonoids contents. BuFI and CoFI had a significant higher phenol and DPPH contents than the CmFI while GiFI and AIFI has lower concentration of the anti-oxidants.

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 a significant increase in the vitamin composition (with the exception of 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 when compared with the CmFI.

**Table1. Anti-nutritional compositions of unfermented 'iru' and spices fortified fermented 'iru'**

Samples	Phytate mg/g	Trypsin inhibitor
UnFI	53.56 <sup>a</sup> ±0.02	33.70 <sup>a</sup> ±1.20
NaFI	25.96 <sup>bc</sup> ±0.03	29.40 <sup>b</sup> ±0.08
BuFI	28.84 <sup>bc</sup> ±0.00	24.68 <sup>e</sup> ±0.12
AIFI	4.53 <sup>e</sup> ±0.58	28.86 <sup>c</sup> ±0.88
GiFI	18.54 <sup>d</sup> ±0.58	30.05 <sup>b</sup> ±0.49

NuFI	20.19 <sup>cd</sup> ±0.00	29.59 <sup>b</sup> ±0.08
GaFI	21.42 <sup>c</sup> ±1.17	33.69 <sup>a</sup> ±0.54
TuFI	53.56 <sup>a</sup> ±1.17	26.71 <sup>cd</sup> ±0.21
GsFI	36.26 <sup>b</sup> ±1.07	31.19 <sup>ab</sup> ±0.12
CoFI	25.96 <sup>bc</sup> ±1.53	26.13 <sup>d</sup> ±0.12s
SkFI	32.55 <sup>a</sup> ±0.00	33.57 <sup>a</sup> ±1.07
CmFI	21.40 <sup>c</sup> ±0.04	31.19 <sup>ab</sup> ±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 <sup>h</sup> ±0.02	60.02 <sup>g</sup> ±1.20	50.0 <sup>g</sup> ±0.12
NaFI	1.01 <sup>e</sup> ±0.03	70.00 <sup>f</sup> ±0.08	71.90 <sup>c</sup> ±0.11
BuFI	0.84 <sup>g</sup> ±0.04	100.77 <sup>a</sup> ±1.07	76.46 <sup>a</sup> ±0.11
AIFI	0.94 <sup>f</sup> ±0.03	75.03 <sup>e</sup> ±0.85	56.72 <sup>f</sup> ±0.42
GiFI	1.01 <sup>e</sup> ±0.01	84.69 <sup>d</sup> ±1.07	66.83 <sup>e</sup> ±0.16
NuFI	1.19 <sup>d</sup> ±0.03	95.71 <sup>b</sup> ±0.85	71.92 <sup>c</sup> ±0.58
GaFI	1.21 <sup>cd</sup> ±0.03	83.64 <sup>d</sup> ±1.07	56.72 <sup>f</sup> ±1.51
TuFI	1.23 <sup>cd</sup> ±0.03	88.62 <sup>c</sup> ±0.86	69.26 <sup>d</sup> ±0.37
GsFI	1.26 <sup>c</sup> ±0.02	87.26 <sup>c</sup> ±0.21	68.63 <sup>d</sup> ±0.42
CoFI	1.43 <sup>b</sup> ±0.01	96.92 <sup>b</sup> ±0.50	73.51 <sup>b</sup> ±0.11
SkFI	1.68 <sup>a</sup> ±0.04	94.96 <sup>b</sup> ±1.07	71.37 <sup>c</sup> ±0.31
CmFI	1.26 <sup>c</sup> ±0.04	87.62 <sup>c</sup> ±0.50	73.50 <sup>b</sup> ±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 =

Tumericfermented iru, GsFI = Grain of selime fermented iru, CoFI = Cocoplum fermented iru, SkFI = Skinplum fermented iru, CmFI = Commercially fermented iru.

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

**Legend:** UnFI = Unfermented iru, NaFI = Naturally fermented iru, StFI = Starter culture fermented iru, AlFI =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 <sup>g</sup> ±0.11
NaFI	11.05 <sup>c</sup> ±0.05
BuFI	12.08 <sup>b</sup> ±0.08

AIFI	9.31 <sup>f</sup> ±0.11
GiFI	11.07 <sup>c</sup> ±0.17
NuFI	12.45 <sup>a</sup> ±0.11
GaFI	10.08 <sup>e</sup> ±0.02
TuFI	9.27 <sup>f</sup> ±0.13
GsFI	9.46 <sup>f</sup> ±0.15
CoFI	10.57 <sup>d</sup> ±0.13
SkFI	10.26 <sup>e</sup> ±0.23
CmFI	12.06 <sup>b</sup> ±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.

## DISCUSSION

Though, there was a reduction in phytic acid during fermentation, the relative 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. That is, the addition of spices might have hindered the phytase activities [16]. While the reduction of phytic acid in samples fortified with alligator pepper might be due to increase in phytase activity during fermentation.

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

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 while the addition of cocoplum led to an increase in total phenol. These might be because these spices have been able to enhance 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 due the fact that the addition of spices reduced the ability of the fermenting organisms in secretion of the enzymes responsible for breakdown of the glycosidic bonds of these antioxidants. This result is in agreement with earlier report in the total phenol content of many plant foods which is proportional to the anti-oxidant capacity of the plant food [17].

The higher value of Vit. A, Vit. B1, Vit. B2, and Vit. B5 in sample fortified with Alligator pepper could be due to the presence of the vitamins in the spice which was 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 with 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 have 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 the level of 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].

## 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 an 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 produced iru. Hence, in fortification of fermented *Parkia biglobosa* seeds to enhance its nutritional composition, alligator pepper, skinplum and nutmeg may be used.

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