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Effect of the Use of Different Concentrations of 'Kuuru' on the Nutritional Quality of Fermented *Parkia biglobosa* Seeds

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Authors' contributions

This work was carried out in collaboration between both authors. Author TRO performed the bench work, statistical analysis, wrote the first draft of the manuscript and managed the literature. Author EYA initiated the study, provided the protocol, managed the analysis of the study and vetted the final manuscript. Both authors read and approved the final manuscript.

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ABSTRACT

The study was carried out to determine the effect of different concentrations of 'kuuru' on the nutritional quality of fermented African locust bean (*Parkia biglobosa*) seeds. The dried seeds were processed into 'iru-pete', either by boiling the seeds with varying concentrations (1:300 to 1:50 w/w) of 'kuuru' or use of starter culture (*Bacillus subtilis* strain BC4333). The fermentation was carried out in an incubator at 35℃ for 36 h. Commercial samples of 'iru-pete' and 'iru-woro' were used as control. The unfermented sample and fermented products were analyzed for sensory properties, proximate composition, concentration of anti-nutritional factors (phytic acid and trypsin inhibitor), anti-oxidants level (phenol, total flavonoids and free radical scavengers), concentration of vitamins (A, B, C, D and E) and protein digestibility. The commercial samples of both 'iru-woro' and 'iru-pete' were more acceptable than the laboratory-fermented products. Similar trends were observed for stickness, texture, colour, odour and overall acceptance. Other proximate parameters (ash, crude fibre and fat) did not show any consistent trend when the concentration of 'kuuru' added increased. The concentrations of phytic acid and trypsin inhibitor in 'iru-pete' produced using 1:300 to 1:50

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(w/w) of 'kuuru', increased from 6.99 mg/g to 10.43 mg/g and 45.51 mg/g to 60.44 mg/g, respectively. Increasing concentration of 'kuuru' decreased the level of antioxidants present in the fermented products from 1:300 w/w (0.64 mg/g to 0.38 mg/g) in total phenol, (0.86 mg/g to 0.22 mg/g) in total flavonoids and (91.75 mg/g to 73.32 mg/g) in DPPH. Increasing concentration of 'kuuru' led to significant reduction in the vitamins A content of the 'kuuru'-fermented products. Similar trend of decrease was observed for vitamins C and E. The *in-vitro* protein digestibility of 'kuuru' fermented products ranged from 39.91% to 36.09%. This research paper confirms that 'kuuru' is not a suitable additive in the production of 'iru-pete'.

Keywords: Parkia biglobosa; fermentation; anti-nutritional factors; anti-oxidants; phytate; vitamins; Tripsin inhibitors.

1. INTRODUCTION

The seeds of *Parkia biglobosa* are traditionally fermented to produce a delicious soup condiment called 'iru' in Nigeria and other West African countries. It is popularly called 'locust bean' in English and 'iru' in Yoruba language [1]. *Parkia biglobosa* seeds are rich in proteins but cannot be consumed in their raw state due to their high level of anti-nutritional factors which are naturally occurring compounds in many tropical plants [2]. The anti-nutritional factors cause poor protein digestibility both in man and animals and have been linked with some terminal diseases like stroke and cancers [2].

Processing like fermentation can be employed to improve digestibility, nutritive value and flavour of the raw seeds [3]. The processing of 'iru' involves soaking of the seeds in water for 15 minutes, boiling under pressure for 3 hours, dehulling of the seeds by rubbing between palms [4]. It also involves boiling of the seeds the second time and fermenting it to produce 'iru'. However, the production of 'iru-pete' involves the addition of 'kuuru', a local softening agent which is a fermented product of ash and dried seeds of *Hibiscus sabdariffa*. It is a local additive and the major reason for its addition in the processing of 'íru' is to soften the boiled seeds during fermentation. It is necessary to confirm the effects of varying concentrations of 'kuuru' on the nutritional quality of 'iru'. Thus, this research investigated the effects of different concentrations of 'kuuru' on the nutritional quality of 'iru'.

2. MATERIALS AND METHODS

2.1 Source of Materials

African locust bean seeds (ALB), 'kuuru' a fermented product of the seeds of *Hibiscus sabdariffa* ('Isapa' seeds) and ash were purchased from retailers at the King's Market Ado – Ekiti, Ekiti State. The 'isapa' seeds were collected from a farmer in Ado – Ekiti, while the ash used for the experiment was produced by burning cashew tree.

2.2 Preparation of Starter Culture

2.2.1 Preparation of strains of *Bacillus subtilis* for 'iru' production

The inocula were prepared by growing the strains of *Bacillus subtilis* in 50 ml Nutrient Broth (NB) in 250 ml conical flasks for 24 hours under agitation (1000 g value) at 35℃. The turbid cultures were centrifuged at 17888 (g value), 4℃ for 10 mins. The supernatant was decanted and the cells pellet was re-suspended in 5ml of sterile distilled water. The cell population was determined by measuring the optical densities (OD) of broth cultures at 540 nm with Pye Unicam SP6-250 visible spectrophotometer. The volume of the inoculum required to inoculate 300g of substrate to give a final inoculation ratio of 10^4 cells per gram of substrate, was calculated [5]

2.3 Laboratory Production of 'iru'

The dried seeds were processed by adopting the method of Ikenebomeh and Kok [4]. Three hundred grams (300 g) each of the seeds of *Parkia biglobosa* were weighed into nine different 1000 ml beakers. The 300 g seeds was boiled under pressure for 1 h, drained, oven dried and labeled as unfermented seeds (UFS). Another 300 g of the seeds was boiled, drained and poured aseptically into a sterile aluminum can and labeled as naturally-fermented sample (F0K). Six sets of 300 g of the seeds were boiled separately for 1h under pressure with the addition of 'kuuru' of varying concentrations

(1:300 to 1:50 w/w), drained and poured aseptically into sterile fermentation cans and labeled as F1K, F2K, F3K, F4K, F5K and F6K, respectively; and incubated at 35℃ for 36 h.

Commercial 'iru-pete' (CIP) and 'iru-woro' (CIW) served as controls. All fermented and unfermented samples were subjected to the following analyses: microbiological (total microbial load and characterization of the isolates), physico-chemical and enzyme assay. All determinations were done in triplicates.

2.4 Sensory Analysis

A semi-trained panel of thirty undergraduate students from Ekiti State University (EKSU), Ado-Ekiti who were already familiar with 'iru' were used to examined all the 'iru' samples on consistency, texture, colour, ammonia flavour, and overall liking. The tests were based on a 9-point Hedonic scale, with 1 being Dislike Extremely and 9 being Like Extremely [5,6]. Approximately 5 g each of the 'iru' samples were served to each individual for assessment. Questionnaire was prepared and distributed to the students.

2.5 Proximate Analysis

The proximate compositions of the fermented and unfermented samples were determined using standard procedures of AOAC [7]. The parameters determined were protein, ash, crude fibre, fat and carbohydrates. The crude protein content was calculated by multiplying the total nitrogen with the factor 6.25, using Kjedahl method [8]; and crude fibre by AOAC [7]. The amount of lipid (oil) was determined, using Soxhlet extraction method; while the ash content was determined by the method of AOAC [7], the carbohydrate content of each sample was determined by difference.

2.6 Determination of Anti-nutritional Factors

2.6.1 Phytic acid

The method of Young and Greaves [9] 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 3 h 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 which contained 0.05 mg of iron (Fe) per ml of $FeCl_3$. The iron equivalent was obtained and the phytate content in mg/100 mg of dried sample was calculated.

2.6.2 Trypsin inhibitor

The trypsin inhibitor activity (TIA) in the sample was determined according to the method of Smith et al. [10]. The digest contained 1.0 g of the sample, 40 µg of trypsin and 2 mg of N-alpha-benzoyl-DL-Arginine-P-nitroanilidehydrochloride (BAPA). The absorbance was read at 410 nm.

2.7 Determination of Anti-oxidants

2.7.1 Total phenol

The total phenol contents of the samples were determined using the method reported by Singleton et al. [11], while total 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. [12] and Gyamfi et al. [13], respectively.

2.8 Determination of Vitamins

Vitamin A was determined by the method of Parrish [14]; vitamin B by the method of Okwo and Josiah [15]; vitamin C by the method of Benderitter et al. [16], while vitamins D and E were determined by the methods of Pearson [17].

2.9 Determination of multi-enzyme *In vitro* Protein Digestibility

The method of Singh and Krikorian [18] was adopted in the determination of multi-enzyme in-vitro protein digestibility of the samples, using procaine pancreatic trypsin as enzyme. The absorbance was read at 700 nm against reagent blank. The standard calibration (STD) curve was prepared using 100 µg/ml of Bovine Serum Albumen (BSA).

2.10 Statistical Analyses

All data obtained were subjected to statistical analysis. Analysis of variance (ANOVA) and Duncan Multiple Range Test (DMRT) packages in SPSS version 15.0 were used.

3. RESULTS

The scores of sensory evaluation of the fermented products and unfermented subsrate are presented in Table 1. There was an increase

in microbial film on the fermented *Parkia biglobosa* as the concentration of 'kuuru' increased and peaked at 'kuuru': substrate ratio of 1:60 w/w. However, the commercial samples of both 'iru-woro' and 'iru-pete' were more acceptable than the Laboratory-fermented products. Similar trend was observed for skickiness, texture, colour, odour and over-all acceptance. Table 2 shows the proximate composition of the fermented products and unfermented substrate. There was increase in the protein content of the fermenting *Parkia biglobosa* seeds as the concentration of 'kuuru' increased from 32.60% (F1K) to 37.44% (F6K). Other proximate parameters (ash, crude fibre and fat did not show any consistent trend when the concentration of 'kuuru' added increased.

Table 3 shows the anti-nutritional factors and anti-oxidants levels of the fermented and unfermented *Parkia biglobosa* seeds. Fermentation led to a significant decrease in the phytic acid level of the products. However, increasing concentrations of 'kuuru' led to a significant increase in the level of the phytic acid of the fermented products. The phytic acid level in the 'kuuru' fermented products F1K, F2K, F3K, F4K, F5K and F6K were 6.99 mg/g 7.41 mg/g, 7.82 mg/g, 9.06 mg/g, 8.93 mg/g and 10.43 mg/g respectively. Fermentation also led to a significant decrease in the level of trypsin inhibitor. The unfermented sample had the highest trypsin inhibitor (64.36 mg/g). Increasing concentration of 'kuuru' led to a signicant increase in the level of trypsin inhibitor present.

Table 1. Sensory evaluation of fermented and unfermented *Parkia biglobosa* seeds with different concentrations of 'kuuru'

Samples	Stickiness	Texture	Colour	Ammonia odour	Overall-acceptance
UFS	2.70^{g}±1.14	2.33^{f}±1.12	2.03^{f}±1.10	2.31^{e}±1.48	2.53^{g}±1.70
F0K	2.97^{f}±1.54	3.13^{e}±1.81	2.47^{f}±1.55	3.17^{d}±2.14	3.00^{g}±2.07
F1K	4.43^{e}±1.52	4.00^{d}±1.20	4.23^{e}±1.94	3.73^{d}±1.39	4.80^{f}±1.58
F2K	5.70^{d}±1.26	5.57^{c}±1.50	5.30^{d}±1.54	5.37^{c}±1.52	5.33^{ef}±1.56
F3K	6.37^{cd}±1.13	6.13^{bc}±1.77	5.87^{cd}±1.72	5.83^{c}±1.58	5.77^{de}±1.63
F4K	6.67^{bc}±1.45	6.13^{bc}±1.68	6.50^{bc}±1.31	6.07^{bc}±1.20	6.23^{cd}±1.38
F5K	7.33^{ab}±1.54	6.87^{ab}±1.89	6.80^{ab}±1.69	6.87^{ab}±1.85	6.73^{bc}±1.57
F6K	6.47^{c}±1.83	6.27^{ab}±1.90	6.47^{bc}±2.06	6.70^{ab}±2.02	6.63^{bc}±1.52
CIP	6.77^{bc}±0.82	7.03^{a}±0.62	6.93^{ab}±0.58	6.90^{ab}±0.85	7.13^{b}±0.57
CIW	7.60^{a}±0.89	7.00^{a}±0.70	7.47^{a}±0.63	7.53^{a}±0.57	7.93^{a}±0.64

Key: UFS=unfermented substrate, F0K= 'iru' produced without 'kuuru', F1K='iru' produced using 1 g of 'kuuru', F2K='iru' produced using 2 g of 'kuuru', F3K= 'iru' produced using 3g of 'kuuru', F4K='iru' produced using 4g of 'kuuru', F5K= 'iru' produced using 5 g of 'kuuru', F6K='iru' produced using 6 g of 'kuuru', CIP= commercial 'iru-pete' and CIW= commercial 'iru-woro'. Values in the same column having the same superscript do not differ at 0.05

Table 2. Proximate composition (%) of fermented and unfermented *Parkia biglobosa* seeds with different concentrations of 'kuuru'

Samples	Protein	Ash	Crude fibre	Fat	Carbohydrate
UFS	29.89^{f}±0.80	0.44^{bcd}±0.02	9.23^{b}±0.34	22.74^{d}±0.46	37.71^{a}±1.15
F0K	32.64^{e}±0.19	1.87^{e}±0.09	5.60^{c}±0.66	27.68^{a}±1.74	31.49^{b}±1.06
F1K	32.60^{e}±0.23	2.35^{c}±0.08	10.20^{a}±0.38	25.93^{abc}±0.30	28.93^{c}±0.57
F2K	33.32^{de}±0.23	2.31^{c}±0.43	9.96^{ab}±0.59	27.72^{a}±1.30	26.03^{d}±1.04
F3K	33.78^{cd}±0.02	3.32^{a}±0.47	10.56^{a}±0.25	25.38^{bc}±0.57	26.96^{cd}±0.47
F4K	34.29^{c}±0.51	2.11^{de}±0.28	10.31^{a}±0.36	24.74^{bcd}±0.57	28.55^{c}±0.73
F5K	35.09^{b}±0.02	2.91^{ab}±0.11	9.87^{ab}±0.44	24.50^{bcd}±0.40	27.33^{c}±0.67
F6K	37.44^{a}±0.00	2.39^{c}±0.076	10.45^{a}±0.23	25.08^{bc}±2.40	24.63^{e}±2.31
CIP	27.89^{g}±0.80	2.21^{de}±0.23	9.87^{ab}±0.44	24.01^{cd}±0.44	35.94^{A}±0.26
CIW	29.43^{f}±0.01	2.81^{bc}±0.43	10.03^{ab}±0.45	26.25^{ab}±0.49	31.48^{b}±1.01

Key: UFS=unfermented substrate, F0K= 'iru' produced without 'kuuru', F1K='iru' produced using 1 g of 'kuuru', F2K='iru' produced using 2 g of 'kuuru', F3K= 'iru' produced using 3 g of 'kuuru', F4K='iru' produced using 4 g of 'kuuru', F5K= 'iru' produced using 5 g of 'kuuru', F6K='iru' produced using 6 g of 'kuuru', CIP= commercial 'iru-pete' and CIW= commercial 'iru-woro' Values in the same column having the same superscript do not differ at 0.05

The trypsin inhibitor increased from 45.51 mg/g (F1K) to 60.44 mg/g (F6K). However, the sample fermented without addition of 'kuuru' (F0K) had the least trypsin inhibitor content of 42.75 mg/g.

Fermentation increased the anti-oxidants level of the fermented product; while increasing concentration of 'kuuru' decreased the level of anti-oxidants present in the fermented products. The total phenol decreased from 0.64 mg/g (F1K) to 0.38 mg/g (F6K); total flavonoids from 0.86 mg/g (F1K) to 0.22 mg/g (F6K); and DPPH decreased from 91.75 mg/g (F1K) to 73.32 mg/g (F6K) The vitamins and the in-vitro protein digestibility of the unfermented and fermented samples are presented in Table 4. Unfermented substrate UFS had the highest vitamin A (2173 mg/g) content, followed by F0K (2043 mg/g), increasing concentration of 'kuuru' decreased significantly the vitamin A content of the 'kuuru' fermented products. Similar trend was observed for vitamin C and E. The sample F0K had the highest vitamin B content (2.30 mg/g) followed by UFS (1.68 mg/g), while F6K had the least vitamin B content (0.12 mg/g). Also, F0K had the highest vitamin D content, followed by UFS, CIW and CIP. However, increasing concentration of 'kuuru' led to a significant decrease in vitamin D content of 'kuuru' added products. The *in-vitro* protein digestibility of the F0K sample was the highest (40.01%), followed by the 'kuuru'-fermented products, which ranged from 39.91% to 36.09% (F1K – F6K) The in-vitro protein digestibility content of CIP and CIW were 35.45% and 37.30%, respectively.

Table 3. Anti-nutritional factors (mg/g) and anti-oxidant level (mg/g) of fermented and unfermented *Parkia biglobosa* seeds with different concentrations of 'kuuru'

Samples	Phytic acid	Trypsin inhibitor	Total phenol	Total flavonoid	DPPH
UFS	9.39^{b}±0.57	64.36^{a}±0.15	0.42^{ef}± 0.05	0.42^{def}±0.03	68.02^{l}±0.09
F0K	6.43^{e}±0.00	42.75^{i}±0.26	0.64^{b}±0.03	0.99^{a}±0.01	92.96^{a}±0.13
F1K	6.99^{d}±0.23	45.51^{h}±0.00	0.61^{b}±0.02	0.86^{abc}±0.02	91.75^{b}±0.03
F2K	7.41^{cd}±0.00	47.48^{g}±0.40	0.54^{c}±0.26	0.70^{bdc}±0.53	90.89^{c}±0.00
F3K	7.82^{c}±0.00	49.61^{f}±0.25	0.47^{def}±0.02	0.70^{bcd}±0.01	89.04^{d}±0.11
F4K	9.06^{b}±0.00	50.30^{e}±0.14	0.42^{ef}±0.01	0.54^{de}±0.03	88.88^{e}±0.00
F5K	8.93^{b}±0.80	51.92^{d}±0.00	0.38^{f}±0.01	0.35^{ef}±0.90	82.72^{g}±1.18
F6K	10.43^{a}±0.48	60.44^{b}±0.30	0.38^{f}±0.14	0.22^{g}±0.00	73.32^{h}±0.06
CIP	9.61^{b}±0.43	59.08^{c}±0.00	0.50^{de}±0.00	0.67^{cd}±0.03	88.09^{ef}±0.03
CIW	6.91^{d}±0.56	43.45^{h}±0.00	0.72^{a}±0.03	1.07^{a}±0.01	94.15^{a}±0.09

Key: UFS=unfermented subsrate, F0K= 'iru' produced without 'kuuru', F1K='iru' produced using 1 g of 'kuuru', F2K='iru' produced using 2 g of 'kuuru', F3K= 'iru' produced using 3 g of 'kuuru', F4K='iru' produced using 4 g of 'kuuru', F5K= 'iru' produced using 5 g of 'kuuru', F6K='iru' produced using 6 g of 'kuuru', CIP= commercial 'iru-pete' and CIW= commercial 'iru-woro'. Values in the same column having the same superscript do not differ at p=0.05

Table 4. Vitamin contents (mg/g) and *in-vitro* protein digestibility (%) of fermented and unfermented *Parkia biglobosa* seeds with different concentrations of 'kuuru'

Samples	Vitamin A mg/g	Vitamin B mg/g	Vitamin C mg/g	Vitamin D mg/g	Vitamin E mg/g	Protein digestibility (%)
UFS	2173^{a}±0.02	1.68^{b}±0.01	0.81^{a}±0.01	3.08^{b}±0.00	2.34^{a}±0.00	35.78^{g}±0.10
F0K	2043^{b}±0.00	2.30^{a}±0.00	0.70^{b}±0.01	3.81^{a}±0.01	2.39^{a}±0.01	40.01^{a}±0.00
F1K	1920^{c}±0.00	1.33^{c}±0.00	0.68^{c}±0.00	1.82^{d}±0.02	1.70^{b}±0.00	39.91^{a}±0.40
F2K	1530^{d}±0.01	0.83^{e}±0.12	0.66^{d}±0.01	1.63^{e}±0.00	0.78^{d}±0.46	38.27^{b}±0.24
F3K	1013^{g}±0.01	0.59^{f}±0.05	0.65^{d}±0.01	1.35^{f}±0.20	0.52^{e}±0.02	37.77^{c}±0.00
F4K	685^{h}±0.00	0.35^{g}±0.01	0.56^{e}±0.00	0.86^{g}±0.00	0.34^{f}±0.00	37.02^{d}±0.03
F5K	290^{i}±0.01	0.27^{g}±0.01	0.32^{h}±0.01	0.63^{h}±0.00	0.16^{g}±0.02	36.59^{e}±0.10
F6K	281^{i}±0.00	0.12^{h}±0.10	0.27^{i}±0.01	0.44^{i}±0.02	0.11^{g}±0.01	36.09^{f}±0.10
CIP	1246^{f}±0.01	0.97^{d}±0.01	0.48^{g}±0.01	2.76^{c}±0.20	1.35^{c}±0.20	35.45^{h}±0.01
CIW	1249^{e}±0.00	1.16^{d}±0.22	0.53^{f}±0.01	2.86^{c}±0.01	0.70^{d}±0.01	37.30^{f}±1.60

Key: UFS=unfermented substrate, F0K= 'iru' produced without 'kuuru', F1K='iru' produced using 1 g of 'kuuru', F2K='iru' produced using 2 g of 'kuuru', F3K= 'iru' produced using 3 g of 'kuuru', F4K='iru' produced using 4g of 'kuuru', F5K= 'iru' produced using 5 g of 'kuuru', F6K='iru' produced using 6 g of 'kuuru', CIP= commercial 'iru-pete' and CIW= commercial 'iru-woro'. Values in the same column having the same superscript do not differ at p=0.05

4. DISCUSSION

The increase in acceptability of the fermented *Parkia biglobosa* as the concentration of 'kuuru' increased might be due to the increase in the softening effect of 'kuuru' on the seeds. The commercial samples of both 'iru-woro' and 'iru-pete' were more acceptable than the Laboratory-fermented products because the 'kuuru' was only responsible for softening of the seeds and not for fermenting the seeds. Similar trend was observed for skickiness, texture, colour, odour and over-all acceptance. The increase in concentration of soluble protein as the concentration of 'kuuru' increased might be attributed to the activities of the proteolytic enzymes which were secreted during fermentation to breakdown the complex proteins into readily digestible forms. The increase in concentration of 'kuuru' that led to significant increase in the level of phytic acid might be due to inhibitory effect of the 'kuuru' on the activity of the phytase enzyme, produced by bacteria during fermentation. The significant reduction in the antioxidants (phenols, total flavonoids and free radical scavengers) levels might be attributed to inhibitory effects of the 'kuuru' on the microbial enzymes that hydrolyze the glycosidic bonds of the poly-phenolics. Also, the reduction in the free radical scavenging ability with increasing concentration of 'kuuru' might be due to inhibitory activities of 'kuuru' on the hydrolytic enzymes [19]. The significant reduction in the protein digestibility might also be attributed to the inhibory effects of 'kuuru' on the proteolytic enzymes which were secreted during fermentation to breakdown the protein into readily digestible forms.

5. CONCLUSION

High concentrations of 'kuuru' (0.33%-2.00%) that led to significant reduction in anti-oxidants and vitamins level; but significant increase in anti-nutritional factors of the fermented *Parkia biglobosa* seeds, is a confirmation that 'kuuru' should not be an additive for the production of 'iru-pete'.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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