Effects of starter culture and different components of ‘kuuru’ on the nutritional quality of fermented *Parkia biglobosa*  

Omodara, T. R.* and Aderibigbe, E. Y.

Department of Microbiology, Faculty of Science, Ekiti State University, Ado-Ekiti, P. M. B. 5363, Ado-Ekiti, Nigeria.

**ABSTRACT**

Iru–pete was produced by fermenting *Parkia biglobosa* cotyledons, using ‘kuuru’, a local softening agent (dried seeds of *Hibiscus sabdariffa* and ash) and a strain of *Bacillus subtilis* group (BC4333), while commercial sample of *iru-pete* served as control. The proximate composition, anti-nutritional factors, antioxidants, protein digestibility and vitamins of the fermented products were determined. There were significant increases in the crude protein, crude ash and crude fat contents of the starter-culture fermented ‘iru’ (F14). However, significant reductions (P=0.05) were found in the protein and crude fibre contents of ‘iru’ fermented with ‘kuuru’ (IFK). Starter culture fermentation enhanced significant reductions in the phytic acid and trypsin inhibitor of the substrate from 9.61 and 63.36 mg/ml to 8.24 and 57.55 mg/ml, respectively (P=0.05). Conversely, there was significant increase (P=0.05) in phenols, total flavonoids and free radical scavengers of the starter culture-fermented product. The addition of ‘kuuru’ and its components during fermentation led to significant decrease (P=0.05) in vitamins A, B, D and E contents in the fermented products. The starter-culture fermented product had the highest in vitro protein digestibility (41.68%); while the least value was recorded in ‘iru’ fermented with dry seeds of *H. sabdariffa* (IFH) (33.29). A similar trend was observed when fermented with the components of ‘kuuru’. Thus, the use of ‘kuuru’ or its components as softening agents are not suitable additives for production of ‘iru-pete’. The research confirmed that the use of *Bacillus subtilis* BC4333 as starter culture enhanced the nutritional value of *iru-pete*.

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**INTRODUCTION**

Fermentation of African locust bean (*Parkia biglobosa*) seeds results in the production of a local soup condiment called ‘iru’ in the Western part of Nigeria. There are two variants of ‘iru’; the hard type, ‘iru-woro’ and the soft type, ‘iru-pete’. *Parkia biglobosa* is a proteinous seed that is rich in protein. Thus the fermented product, serves as a cheap source of protein for people in the rural communities, whose protein intake is low due to the high cost of animal protein sources (Oyeyiola, 1988).

The traditional method of preparing the soft-type of ‘iru’ involves boiling the seeds for 12-24 h to soften the hard testa and dehulling using sand abrasive. The seed-coats are washed off using perforated calabash. The clear split, cotyledons are boiled for 2 h (Odunfa 1981). Over the years, local women produced ‘iru-pete’ after adding ‘kuuru’ a local softening agent. The ‘kuuru’ is a fermented product of dried seeds of *Hibiscus sabdariffa* (local name: ‘isapa’) and soft wood ash.

This research was carried out to compare the nutrient...
value of ‘iru-pete’ produced from ‘kuuru’ and that produced from starter culture of *Bacillus subtilis* (BC4333). A strain of *B. subtilis* (BC4333) had been previously used by Aderibigbe et al. (2014) to produce ‘iru-pete’ without the addition of ‘kuuru’.

**MATERIALS AND METHODS**

**Source of materials**

The African locust bean (*Parkia biglobosa*) seeds were purchased from Odo-Ado Market, Ado-Ekiti, Ekiti State; while the commercial samples of ‘iru-pete’ and ‘iru-woro’ were purchased from Osele Market in Ikare-Akoko, Ondo State. ‘Kuuru’ was purchased from a retailer at the King’s Market, Ado-Ekiti, Ekiti State. The *H. sabdariffa* seeds were collected from a farmer, while the ash used for the experiment was obtained by burning dried cashew tree branches. Pure culture of *B. subtilis* (strain BC4333) was obtained from the stock cultures kept in the Department of Microbiology, Ekiti State University, Ado-Ekiti, Ekiti State.

**Preparation of starter culture**

The starter culture was prepared by inoculating the *B. subtilis* strain BC4333 grown on nutrient agar (NA) plate into 50 ml sterile nutrient broth (NB) in 250 ml conical flask and was incubated at 35°C (with agitation of 200 rpm) for 24 h. The culture was centrifuged at 10,000 rpm (4°C) for 10 min. The supernatant was decanted and the cells pellets were re-suspended in 5 ml of sterile distilled water. The cell population was determined by measuring the optical densities of the suspension at 540 nm, using a Pye Unicam SP6-250 visible spectrophotometer. The volume of the cells suspension required to inoculate 300 g of substrate to give a final inoculation ratio of 10⁴ cells per gram of substrate was calculated based on the hypothesis that an ODS40 nm of 1.0 is equivalent to 10⁷ cells/ml of *B. subtilis* (Aderibigbe et al., 2014). The volume of the culture was made up to 1 ml in a sterile vial tube using sterile water.

**Laboratory production of ‘iru’**

The method of Ikenebomeh and Kole (1984) was adopted. The seeds were soaked in water for 15 min, boiled using pressure pot for 2 h and dehulled to remove the testa. Three hundred grams (300 g) each of the cotyledons were weighed into six different 1L-beakers. The 300 g cotyledons in first beaker was poured into pressure pot and boiled for 1 h, drained, oven-dried at 75°C for 24 h and was labeled as (UFS) unfermented substrate. The 300 g cotyledons in beaker 2 was also poured into pressure pot and boiled for 1 h, drained and aseptically poured into a sterile of 10 cm × 20 cm × 10 cm rectangular-shaped aluminum fermenting can and was labeled as naturally fermented ‘iru’ (NFL). Five grams (5 g) each of finely ground *H. sabdariffa* seeds, ash, and ‘kuuru’ were added to cotyledons in beakers 3, 4 and 5, respectively, poured into separate pressure pots, and was boiled at 121°C for 1 h. After boiling, it was poured aseptically into sterile fermenting cans of the same dimension used above and they were labeled as; IFH (‘iru’ fermented with *Hibiscus sabdariffa* seeds), IFA (‘iru’ fermented with ash) and IFK (‘iru’ fermented with ‘kuuru’), respectively. The sixth batch of 300 g cotyledons was also boiled under pressure for 1 h, drained and poured aseptically into sterile aluminum fermenting can. It was inoculated with 1.0 ml of the starter culture *B. subtilis* BC4333and labeled as FBC433. All the samples were fermented at 35°C for 36 h. Commercial sample of ‘iru-pete’ (CIP) served as control.

**Proximate analysis**

The proximate compositions of the fermented and unfermented samples were determined using standard procedures of AOAC (2000). The parameters determined were protein, ash, crude fibre, fat and their carbohydrate. The crude protein content was calculated by multiplying the total nitrogen with the factor 6.25, using Kjedahl method (Joslyn, 1970); and crude fibre by AOAC (2000). The amount of lipid (oil) was determined, using Soxhlet extraction method; while the ash content was determined by the method of AOAC (2000), and the carbohydrate content of each sample was determined by difference.

**Determination of anti-nutritional factors**

**Phytic acid**

The method of Young and Greaves (1940) 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. This was titrated against ferric chloride solution which contained 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.

**Trypsin inhibitor**

The trypsin inhibitor activity (TIA) in the sample was
determined according to the method of Smith et al. (1980). 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.

**Determination of anti-oxidants**

**Total phenol**

The total phenol contents of the samples were determined using the method reported by Singleton et al. (1999), 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. (2005) and Gyamfi et al. (1999), respectively.

**Determination of vitamins**

Vitamin A was determined by the method of Parrish (1977); vitamin B by the method of Okwo and Josiah (2006); vitamin C by the method of Benderitter et al. (1998), while vitamins D and E were determined by the methods of Pearson (1976).

**Determination of multi-enzyme in vitro protein digestibility**

The method of Singh and Krikorian (1982) was adopted in the determination of multi-enzyme in vitro protein digestibility of the samples, using procain 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).

**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.

**RESULTS**

There was significant increase in the ash content of ‘iru’ fermented with ash (3.64%) and ‘iru’ produced using B. subtilis BC4333 (3.29%) during fermentation. Crude fibre increased from 9.23% in UFS to 10.81% in IFA. However, the percentage of crude fibre in CIP, IFH seeds and ‘iru’ produced using B. subtilis strain BC4333 (F14) were not significantly different from UFS. Fermentation significantly led to an increase in fat contents of the fermented products. However, fermentation decreased significantly the carbohydrate contents of the fermented products (Table 1).

Table 2 shows the anti-nutritional factors and the anti-oxidants levels. The phytic acid decreased significantly from 9.61 mg/ml in UFS to 9.06 mg/g in IFA and to 6.43 mg/g in ‘iru’ fermented with B. subtilis strain BC4333 (F14). Similarly, trypsin inhibitory activity also decreased significantly from 63.36 to 57.55 mg/g and 42.75 mg/g in ‘iru’ fermented using B. subtilis strain BC4333 (F14). The results of total flavonoids showed that ‘iru’ fermented with B. subtilis strain BC4333 (F14) and CIP, had the highest values of 1.09 and 1.07 mg/g, respectively. However, the total flavonoids content of naturally fermented product (NFP) and IFK were not significantly different from each other. Also, CIP, IFH and IFA, had total phenol concentrations which were not significantly different from each other. Unfermented samples (UFS) had the lowest total flavonoids value of 0.42 mg/g. The diphenyl picrylhydrazyl (DPPH) radical scavengers was higher in IFH seeds (94.68 mg/g) than other samples, followed by ‘iru’ fermented with B. subtilis strain BC4333 (F14) (94.15 mg/g), NFI (90.89 mg/g), IFK (90.72 mg/g), but was the least in UFS (68.02 mg/g).

The vitamins and the in vitro protein digestibility of the unfermented and fermented samples are presented in Table 3. The ‘iru’ fermented with B. subtilis strain BC4333 (F14) had the highest values of vitamin A, B, C and E while IFK had the least value for all the vitamins considered.

The in vitro protein digestibility of the unfermented and fermented ‘iru’ samples showed that ‘iru’ fermented with B. subtilis strain BC4333 (F14) 41.69% had the highest percentage, followed by NFS (40.06%). However, ‘iru’ fermented with IFK (933.29%) had the least percentage of in vitro protein digestibility.

**DISCUSSION**

The percentages of protein and fat observed in the NFP, ‘iru’ fermented using B. subtilis strain BC4333 (F14) and IFH seeds were higher than the UFS. The increase in protein and fat might be due to the utilization of the carbohydrate by the fermenting organisms for metabolism. The observed decrease in protein content of CIP and IFK may be attributed to the utilization of the microorganisms that utilize the protein for metabolism. During fermentation, microorganisms hydrolyze protein to release free amino acids, which can be used for synthesis of new protein (Ene-Obong and Obizoba,
The reduction in the ash content of IFK, CIP, IFH seeds and NFP may be due to leaching of solute inorganic salts into the processing water during boiling of the substrate; and this agrees with the report of Osman (1996). The decrease in ash may also be attributed to possible losses of the dry matter and volatile compounds which normally occur during fermentation (Nmam and Obiakor, 2003). The increase in the crude fibre content of IFA, CIP and IFH seeds may be attributed to the boiling of the samples, which might have led to the cleavage

Table 1. Proximate composition of the fermented and unfermented samples.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein (g/100g)</th>
<th>Ash (g/100g)</th>
<th>Crude fibre (g/100g)</th>
<th>Fat (g/100g)</th>
<th>Carbohydrate (g/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFS</td>
<td>29.43±0.01</td>
<td>2.43±0.03</td>
<td>9.23±0.34</td>
<td>22.74±0.46</td>
<td>29.13±0.66</td>
</tr>
<tr>
<td>IFH</td>
<td>35.89±0.23</td>
<td>2.16±0.12</td>
<td>9.54±0.59</td>
<td>29.02±0.22</td>
<td>13.32±0.18</td>
</tr>
<tr>
<td>IFA</td>
<td>28.62±0.62</td>
<td>3.64±0.19</td>
<td>10.81±0.35</td>
<td>24.61±0.35</td>
<td>22.43±0.31</td>
</tr>
<tr>
<td>IFK</td>
<td>25.04±0.57</td>
<td>2.39±0.08</td>
<td>7.94±0.56</td>
<td>25.64±0.39</td>
<td>29.80±0.77</td>
</tr>
<tr>
<td>NFP</td>
<td>36.28±0.63</td>
<td>1.87±0.09</td>
<td>7.51±0.44</td>
<td>29.10±0.56</td>
<td>19.28±1.10</td>
</tr>
<tr>
<td>F14</td>
<td>36.28±0.17</td>
<td>3.29±0.44</td>
<td>9.40±0.41</td>
<td>27.44±0.40</td>
<td>18.16±1.10</td>
</tr>
<tr>
<td>CIP</td>
<td>27.56±1.80</td>
<td>2.21±0.23</td>
<td>9.87±0.44</td>
<td>24.10±0.44</td>
<td>27.66±0.96</td>
</tr>
</tbody>
</table>

UFS, Unfermented sample; IFH, 'iru' fermented with Hibiscus sabdariffa seeds; IFA, 'iru' fermented with ash; IFK, 'iru' fermented with 'kuuru'; NFP, fermented product; F14, 'iru' fermented with Bacillus subtilis strain BC4333; CIP, commercial 'iru-pete'. Values in column having the same superscript do not differ significantly at 0.05.

Table 2. Anti-nutritional factors and anti-oxidant levels of the fermented and unfermented sample of P. biglobosa.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Phytic acid (mg/ml)</th>
<th>Trypsin inhibitor (mg/ml)</th>
<th>Total phenol (mg/ml)</th>
<th>Total flavonoids (mg/ml)</th>
<th>Free radical scavenger (mg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFS</td>
<td>9.61±0.47</td>
<td>63.36±0.15</td>
<td>0.42±0.05</td>
<td>0.42±0.03</td>
<td>68.02±0.09</td>
</tr>
<tr>
<td>IFH</td>
<td>7.69±0.48</td>
<td>49.70±0.15</td>
<td>0.43±0.03</td>
<td>0.61±0.01</td>
<td>94.68±0.09</td>
</tr>
<tr>
<td>IFA</td>
<td>9.06±0.00</td>
<td>46.12±0.29</td>
<td>0.42±0.05</td>
<td>0.61±0.09</td>
<td>74.03±0.01</td>
</tr>
<tr>
<td>IFK</td>
<td>8.24±0.00</td>
<td>57.55±3.00</td>
<td>0.56±0.05</td>
<td>0.85±0.02</td>
<td>90.72±0.12</td>
</tr>
<tr>
<td>NFP</td>
<td>7.41±0.00</td>
<td>50.30±0.14</td>
<td>0.50±0.00</td>
<td>0.86±0.02</td>
<td>90.89±0.00</td>
</tr>
<tr>
<td>F14</td>
<td>6.43±0.03</td>
<td>42.75±1.88</td>
<td>0.83±0.04</td>
<td>1.09±0.01</td>
<td>94.15±0.09</td>
</tr>
<tr>
<td>CIP</td>
<td>6.99±0.23</td>
<td>47.48±0.40</td>
<td>0.61±0.02</td>
<td>1.07±0.01</td>
<td>84.71±0.02</td>
</tr>
</tbody>
</table>

UFS, Unfermented sample; IFH, 'iru' fermented with Hibiscus sabdariffa seeds; IFA, 'iru' fermented with ash; IFK, 'iru' fermented with 'kuuru'; NFP, fermented product; F14, 'iru' fermented with Bacillus subtilis strain BC4333; CIP, commercial 'iru-pete'. Values in column having the same superscript do not differ significantly at 0.05.

Table 3. Vitamin contents and percentage protein digestibility of the fermented and unfermented sample of Parkia biglobosa.

<table>
<thead>
<tr>
<th>Sample</th>
<th>Vitamin A (mg/ml)</th>
<th>Vitamin B (mg/ml)</th>
<th>Vitamin C (mg/ml)</th>
<th>Vitamin D (mg/ml)</th>
<th>Vitamin E (mg/ml)</th>
<th>Protein digestibility (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>UFS</td>
<td>2173±23.09</td>
<td>1.69±0.01</td>
<td>0.23±0.01</td>
<td>3.08±0.00</td>
<td>1.70±0.00</td>
<td>35.78±0.09</td>
</tr>
<tr>
<td>IFH</td>
<td>1697±11.5</td>
<td>1.18±0.05</td>
<td>0.79±0.01</td>
<td>2.31±0.12</td>
<td>1.11±0.05</td>
<td>33.29±0.00</td>
</tr>
<tr>
<td>IFA</td>
<td>953±5.77</td>
<td>0.49±0.03</td>
<td>0.53±0.01</td>
<td>0.96±0.06</td>
<td>0.38±0.02</td>
<td>36.29±0.15</td>
</tr>
<tr>
<td>IFK</td>
<td>386±5.77</td>
<td>0.23±0.03</td>
<td>0.49±0.01</td>
<td>0.75±0.02</td>
<td>0.24±0.02</td>
<td>39.76±0.00</td>
</tr>
<tr>
<td>NFP</td>
<td>2043±5.77</td>
<td>1.43±0.00</td>
<td>0.70±0.01</td>
<td>1.63±0.00</td>
<td>1.45±0.06</td>
<td>40.06±0.15</td>
</tr>
<tr>
<td>F14</td>
<td>2166±11.5</td>
<td>2.31±0.01</td>
<td>0.81±0.01</td>
<td>2.86±0.02</td>
<td>2.43±0.00</td>
<td>41.86±0.09</td>
</tr>
<tr>
<td>CIP</td>
<td>1246±11.5</td>
<td>0.97±0.10</td>
<td>0.48±0.01</td>
<td>2.86±0.31</td>
<td>1.53±0.18</td>
<td>35.45±0.09</td>
</tr>
</tbody>
</table>

UFS, Unfermented sample; IFH, 'iru' fermented with Hibiscus sabdariffa seeds; IFA, 'iru' fermented with ash; IFK, 'iru' fermented with 'kuuru'; NFP, fermented product; F14, 'iru' fermented with Bacillus subtilis strain BC4333; CIP, commercial 'iru-pete'. Values in column having the same superscript do not differ significantly at 0.05.
of the carbohydrate leakages, hereby facilitating the easy extraction of the oil by extracting solvent. The reduction in crude fibre content due to fermentation in IFK, NFP and IFH seeds might be due to the activities of fermenting micro floral to hydrolyze and metabolize them as carbon source in other to synthesize cell biomass (Ene-Obong and Obizoba, 1996).

The phytic acid content of fermented African locust bean samples reduced significantly as a result of fermentation. The loss in phytic acid could be attributed to leaching of acid during soaking, washing and lactic acid fermentation of the cotyledons. The reduction in phytic acid level could also be due to the activity of the enzyme and phytase produced by fermenting bacteria to degrade the phytic acid or the complex formed by them (Deacon, 2005). Phytate are heat-stable thereby making several minerals unavailable for absorption. They are also known to chelate some divalent metal ions, notably Ca$^{2+}$, Mg$^{2+}$, Zn$^{2+}$ and Mn$^{2+}$, thus making them metabolically unavailable (Deacon, 2005). The decrease in trypsin inhibitor of African locust beans by fermentation may be attributed to the leaching during soaking, heat treatment during boiling and also by the action of microorganisms during fermentation. Similar result was reported by other workers on African locust beans and chick pea (El-Adawy, 2002).

The observed increase in phenolic contents of the condiment after fermentation may be due to the activities of microbial enzymes to hydrolyze glycosidic bonds of the phenolics (Oboh and Rocha, 2007a). The total phenol distribution in both the fermented and unfermented products is in agreement with earlier reports on total phenols distribution for some commonly consumed fruits, vegetables and some varieties of hot peppers in which free-phenolics out-numbered the bond-phenolics (Oboh and Rocha, 2007a, b). The reduction in the total phenol due to increasing addition of ‘kuuru’ or its contents may also be due to the ability of fermenting organisms to utilize them for metabolism. The reducing ability of phenolics extract from ‘iru’ has ability to reduce Fe$^{3+}$ to Fe$^{2+}$; this means that the total phenol has a significant higher reducing power. This result is in agreement with earlier report in the total phenol content of many plant foods and it is proportional to the anti-oxidant capacity of the plant food (Oboh and Akindahunsi, 2004). The fermented products had a significantly high free radical scavenging ability than those from the unfermented samples. This finding agrees with earlier report of Vattam et al. (2004). The increased free radical scavenging level of fermented product is that microbial enzymes may have hydrolyze glycosidic bonds to release free radical scavenging with more available functional groups for anti-oxidants activity (Oboh and Akindahunsi, 2004).

Fermentation was found to create a significant effect on in vitro protein digestibility of ’iru’. During fermentation, the proteolytic enzymes secreted by the fermenting organism increased the protein digestibility. Similar findings showed that microorganisms were found to produce proteolytic enzymes during fermentation, which dissociates and degrade proteins, rendering them more accessible to protease, and hence increase digestibility (Aderibigbe et al., 1990).

The high value of vitamin A in the unfermented sample may be due to the fact that the fermenting organisms have not metabolized the vitamin and that the enzymes from the fermenting organisms have not acted on the vitamin A. The higher value of vitamin A, B, C, and E of ’iru’ produced using BC4333 (F14) strain, may be due to the activities of the starter culture in fermenting the substrate, which breaks down some bonds in the fermenting beans, leading to increase in the vitamin content. The decrease in the values of vitamin D during fermentation may be due to the metabolic activities of the fermenting organisms or the vitamins might have been converted to other useful biochemical molecules, such as proteins (Isler et al., 1967).

IFK had the least values of vitamins A, B, C, D and E. This may be due to the fact that the fermenting organism metabolized them, or the leaching away of the vitamins or the vitamins had been destroyed by the alkali that was present in ‘kuuru’. ‘Kuuru’ and its components drastically reduced the nutritional quality of ’iru-pete’, while B. subtilis BC4333 increased the nutritional qualities. Hence, B. subtilis BC4333 can be recommended for local production of ’iru-pete’ by rural women.

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