EFFECTS OF THE USE OF STARTER CULTURE ON THE QUALITY OF FERMENTED PARKIA BIGLOBOSA

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ABSTRACT

The effects of the use of starter-culture on the quality of fermented Parkia biglobosa (‘iru’) were investigated. ‘Iru’ was prepared using fourteen (14) strains of Bacillus subtilis isolated from commercial samples of ‘iru’ as starter cultures. The physicochemical properties, sensory assessment and proximate composition of the unfermented samples, fermented products and commercial samples were determined. The pH values of the starter culture-fermented products ranged between 7.29 and 7.58. However, there was inverse correlation between the total titratable acidity (TTA). The moisture content of the unfermented sample (UFS) 50.67% was higher than that of some of the starter culture-fermented products which ranged between 46.67% and 59.33% while the moisture contents of commercial ‘iru-woro’ (CIW) and commercial ‘iru-pete’ (CIP) were 56.67% and 57.33% respectively. Sensory assessment indicated that the use of Bacillus subtilis strains 2B and 3A as starter cultures for production of ‘iru’ gave more acceptable products than commercial ‘iru-woro’ with values 8.50±0.57a and 8.22±0.06ab respectively.

However, products from Bacillus subtilis strain 6C and unfermented samples (UFS) were rated least. The proximate composition result showed that fermentation increased the protein content from 31.26% in unfermented samples to 43.44% and 42.61% in products of Bacillus subtilis strains 3A and 2B respectively. Fat and ash contents of the products also increased in similar trend. However, there was a decrease in carbohydrate and crude fibre contents of the substrate during fermentation. Hence strains 3A and 2B may be recommended for use as starter cultures in commercial production of ‘iru-woro’ and strain BC4333 for commercial production of ‘iru-pete’

KEYWORDS: Bacillus, Fermentation, Starter Culture, Parkia biglobosa

INTRODUCTION

African locust bean (Parkia biglobosa) belongs to the family leguminaceae. The pods are flat, large, irregular clusters, from which the seeds are obtained (Omafuvbe et al., 2004). The species of the genus include Parkia filicoidea, Parkia bicolor and Parkia clappertoniana (Oke, 1978). African locust bean is extremely hard and inedible in the raw state. It is commonly processed and fermented into a tasty product, known by several names in West Africa. The product is called ‘iru’ in the Yoruba – speaking areas of Nigeria. ‘Iru’ is used as a condiment in numerous dishes. However, the smell of the fermented product sometimes becomes undesirable; and as such makes the product unpopular among some urban dwellers.

The African locust bean seed is rich in protein (Oyenuga, 1968; Odunfa, 1981), thus the fermented product can serve as a cheap source of protein for people whose protein intake is low, due to the high costs of animal protein. There are two variants of ‘iru’, ‘iru-woro’ and ‘iru-pete’. In traditional methods of manufacture, the fermentation of legumes seed is achieved by indigenous microflora or addition of fermented materials from previous production (Achi, 2005). Virtually all the fermentation processes are at local level and rudimentary utensils are used. The quality vary considerably, shelf-life is very short, while some are sources of pathogenic organisms. Thus, it may be assumed that undefined starter culture have
traditionally been employed in the manufacturing of the food product (Suberu and Akinyanju, 1996; Omafuvbe et al, 2002; Dakwa et al, 2005).

There is need to apply modern biotechnological techniques, like the use of starter culture in improving traditional food processing technology. Starter culture has been found to reduce fermentation time as well as guarantee product safety (Achi, 2005). The use of proven strains in effecting the fermentation will greatly contribute to product of better quality. Taking into consideration the increasing demand, particularly by urban population in Nigeria, there are prospects for industrialization of traditionally fermented condiments. Commercial availability and ready-to-use fermented products saves much labour and time in household (Nout and Sarker, 1999). This research aims at elucidating the effect(s) of using starter cultures during fermentation of *Pakia biglobosa* to produce ‘iru’.

**MATERIALS AND METHODS**

**Source of Materials**

African locust bean seeds were purchased from retailers at the King’s Market, Ado-Ekiti, Ekiti State, Nigeria. Starter cultures used were pure cultures of *Bacillus subtilis* group which were previously isolated from commercial samples of ‘iru’ and kept as stock cultures in the Department of Microbiology, Ekiti State University, Ado-Ekiti.Ekiti State. The commercial ‘iru’ samples were purchased from Osele Market in Ikare Akoko, Ondo State.

**Preparation of Starter Cultures**

The innocula were prepared by growing 14 strains of *Bacillus subtilis* in 50ml Nutrient Broth (NB) in 250ml conical flasks for 24 hours under agitation (200rpm) at 35°C. The turbid cultures were centrifuged at 10,000rpm, 4°C for 10mins. The supernatant was decanted and the cell pellets were re-suspended in 5ml of sterile distilled water. The cell population was determined by measuring the optical densities of broth cultures at 540nm 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.

**Starter-Culture Fermentation**

The dried African locust bean seeds were processed by modifying the method of Ikenebomeh and Kok (1984). The dried seeds were hand picked to remove dirt and boiled under pressure for 3 hours. The cooked seeds were dehulled and washed thoroughly to remove the testa. The cotyledons were boiled again for 1 hour. Three hundred grams (300g) of the boiled substrate each were weighed separately into fifteen sterile baking pans. One millimeter (1ml) of suitably dialyzed starter cultures was used to inoculate each of the baking pans containing the substrate; while un-inoculated substrate served as control.

The inoculated substrate were mixed using flamed spatula and incubated at 35°C for 36h. Fermented samples and control were analyzed for physic-chemical properties, sensory evaluation and proximate composition.

**pH Determination**

Five grams (5g) of each sample was blend with 20ml of distilled using a warring blender to produce an homogenate. The pH of each homogenate was determined with a PY8E Unicam pH meter (Model PW9409).

**Total Titratable Acidity**

The suspension from the pH determination was filtered using No 1 Watman filter paper. 20ml of the filtrate was titrated against 0.1M NaOH using phenolphthalein. Titratable acidity was determined as reported by Joslyn (1970).
Moisture Content Determination

The moisture content was quantified using the method described by the Association of Analytical Chemist, AOAC, 2000.

Sensory Analysis

A semi-trained panel of thirty undergraduate students from Ekiti State University, Ado-Ekiti (EKSU), who were already familiar with ‘iru’ were used to examine all the ‘iru’ samples on consistency, texture, colour, ammonia flavor, and overall liking, using the method describe by Tanya et al.,(1997).

Chemical Analysis

The proximate chemical compositions were evaluated by the method described by the Association of Analytical Chemist, AOAC, 2000.

Statistical Analysis

All data obtained were expressed as the mean ± standard deviation (SD).The significance of difference was tested using ANOVA (Analysis of variance) and Duncan Multiple Range Test (DMRT) were used under SPSS15.0 package.

RESULTS

Figures 1 and 2 show the pH and total titratable acidity (TTA) of the ‘iru’ samples respectively. The pH values of the products ranged from 7.01 to 7.58, while the unfermented substrate had slight acidic pH of 6.39. The starter culture-fermented products had the titratable acidity (TTA) values, which ranged between 0.97×10^{-2} and 1.31×10^{-2} N, while the unfermented substrate had the highest titratable acidity of 1.35×10^{-2}. However CIW and CIP had TTA values of 0.81×10^{-2} and 0.85×10^{-2} respectively. The moisture contents of the samples are shown in figure 3 below. Result showed that ‘iru’ produced using Bacillus subtilis strain BC4333 had the highest moisture content of 59.3%, followed by commercial ‘iru-pete’ and commercial ‘iru-woro’ which had 57.6% and 56.3% respectively. The moisture content of the unfermented sample was 50.67%.

![Figure 1: pH Content of the ‘iru’ Samples](image)

**Key:** A=sample fermented with strain 8B, B= sample fermented with strain 1A, C = sample fermented with strain 9B,
D= sample fermented with strain 10B, E = sample fermented with strain 2B, F= sample fermented with strain 7A,
G= sample fermented with strain 3A, H= sample fermented with strain 4A, I= sample fermented with strain 6A,
J= sample fermented with strain 3B, K= sample fermented with strain = 9A, L= sample fermented with strain 5A,
M= sample fermented with strain 6C, N= sample fermented with strain BC4333, O=Unfermented sample,
P=commercial ‘iru-woro’, Q-commercial ‘iru-pete’.
Figure 2: Total Titratable Acidity (TTA) of the ‘iru’ Samples


Figure 3: Moisture Content of the ‘iru’ Samples


The sensory evaluation results of the samples are presented in Table 1. The ‘iru’ produced using *Bacillus subtilis* strain 3A and strain 2B were rated best in terms of consistency, followed by commercial ‘iru-woro’ and ‘iru’ produced using *Bacillus subtilis* strain BC4333, while the unfermented substrate was rated least. Similar trend was also observed for the levels of ammonia odour perceived. Amongst the starter culture-fermented samples, the *Bacillus subtilis* strain 3A and strain 2B products were rated best in the overall-liking, followed by sample produced using *Bacillus subtilis* strain BC4333. Commercial ‘iru-woro’ and commercial ‘iru-pete’ were also better rated in the overall-likening.

Table 2 shows the proximate composition of the ‘iru’ samples. The ‘iru’ produced using *Bacillus subtilis* strain 3A and strain 2B had the highest protein content of 43.44% and 42.81% respectively, followed by ‘iru’ produced using *Bacillus subtilis* strain BC4333of 39.40%. The protein contents of commercial ‘iru-woro’ and commercial ‘iru-pete’ were
40.51% and 38.13% respectively. The protein content of the remaining starter culture-fermented samples ranged between 33.32% – 37.42%. The unfermented sample had the least protein content of 31.26%. The unfermented sample had the highest ash content of 4.46% followed by ‘iru’ produced using Bacillus subtilis strain 6C 3.67%; While the products by Bacillus subtilis BC4333 had the least ash content of 2.22%.

The fat contents of ‘iru’ produced using Bacillus subtilis strain 3A and strain 2B were statistically higher than the other samples. The fat content in commercial ‘iru-woro’ and commercial ‘iru-pete’ were 28.86% and 26.98% respectively, though the data were not statistically different from each other. This was followed by fat contents of ‘iru’ produced using Bacillus subtilis strain BC4333 which was 26.25%. The unfermented sample had the least fat content, while the fat content of the other starter culture-fermented samples had values that ranged between 20.8% and 26.25%. The crude fibre content of most of the starter culture-fermented ‘iru’ samples were found to be higher than that of commercially prepared samples. The unfermented sample had the highest crude fibre content. The carbohydrate content of the starter culture-fermented samples were found to be higher than that of commercial ‘iru’ samples. The carbohydrate content of unfermented sample was in the same range of the starter culture fermented sample.

**Table 1: Sensory Analysis of the Starter Culture-Fermented and Unfermented Samples of Parkia biglobosa**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Consistency</th>
<th>Texture</th>
<th>Colour</th>
<th>Odor</th>
<th>Overall Likening</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>7.0±1.20</td>
<td>6.70±1.12</td>
<td>6.83±0.95</td>
<td>5.83±1.02</td>
<td>B</td>
</tr>
<tr>
<td>B</td>
<td>6.93±0.64</td>
<td>6.17±0.79</td>
<td>6.43±1.01</td>
<td>6.20±0.55</td>
<td>6.47±0.82</td>
</tr>
<tr>
<td>C</td>
<td>3.87±1.5</td>
<td>5.17±1.45</td>
<td>4.77±0.97</td>
<td>4.57±1.36</td>
<td>5.30±0.70</td>
</tr>
<tr>
<td>D</td>
<td>4.33±1.21</td>
<td>5.17±1.45</td>
<td>5.30±1.44</td>
<td>5.03±1.22</td>
<td>5.37±1.10</td>
</tr>
<tr>
<td>E</td>
<td>8.07±0.87</td>
<td>7.83±0.87</td>
<td>7.87±0.86</td>
<td>7.87±0.78</td>
<td>8.20±0.66</td>
</tr>
<tr>
<td>F</td>
<td>2.67±0.88</td>
<td>2.73±0.87</td>
<td>2.57±0.82</td>
<td>2.63±0.77</td>
<td>2.73±0.83</td>
</tr>
<tr>
<td>G</td>
<td>8.47±0.68</td>
<td>8.43±0.63</td>
<td>8.47±0.57</td>
<td>8.47±0.73</td>
<td>8.50±0.57</td>
</tr>
<tr>
<td>H</td>
<td>6.67±0.88</td>
<td>6.37±0.77</td>
<td>6.07±0.91</td>
<td>6.03±0.76</td>
<td>6.57±0.73</td>
</tr>
<tr>
<td>I</td>
<td>3.00±0.59</td>
<td>2.30±0.60</td>
<td>2.53±0.63</td>
<td>2.07±0.54</td>
<td>2.97±0.49</td>
</tr>
<tr>
<td>J</td>
<td>2.53±0.68</td>
<td>2.70±0.46</td>
<td>2.37±0.56</td>
<td>2.33±0.66</td>
<td>2.43±0.68</td>
</tr>
<tr>
<td>K</td>
<td>2.30±0.60</td>
<td>2.13±0.51</td>
<td>2.47±0.63</td>
<td>3.33±1.03</td>
<td>2.90±0.92</td>
</tr>
<tr>
<td>L</td>
<td>4.47±0.73</td>
<td>4.00±0.77</td>
<td>3.83±0.87</td>
<td>3.20±0.85</td>
<td>4.07±0.69</td>
</tr>
<tr>
<td>M</td>
<td>1.53±0.63</td>
<td>1.63±0.62</td>
<td>1.77±0.50</td>
<td>1.30±0.47</td>
<td>1.40±0.56</td>
</tr>
<tr>
<td>N</td>
<td>7.60±0.07</td>
<td>7.0±0.62</td>
<td>6.93±0.11</td>
<td>6.93±0.85</td>
<td>7.13±0.71</td>
</tr>
<tr>
<td>O</td>
<td>1.73±0.52</td>
<td>1.70±0.47</td>
<td>1.67±0.48</td>
<td>1.53±0.51</td>
<td>1.77±0.43</td>
</tr>
<tr>
<td>P</td>
<td>7.60±0.90</td>
<td>7.0±0.13</td>
<td>7.57±0.62</td>
<td>7.53±0.57</td>
<td>7.93±0.64</td>
</tr>
<tr>
<td>Q</td>
<td>5.60±0.50</td>
<td>3.57±0.10</td>
<td>5.43±0.50</td>
<td>6.50±0.51</td>
<td>5.50±0.57</td>
</tr>
</tbody>
</table>

**Key:**
- A = sample fermented with strain 8B, B = sample fermented with strain 1A, C = sample fermented with strain 9B,
- D = sample fermented with strain 1B, E = sample fermented with strain 2B, F = sample fermented with strain 7A,
- G = sample fermented with strain 3A, H = sample fermented with strain 4A, I = sample fermented with strain 6A,
- J = sample fermented with strain 3B, K = sample fermented with strain = 9A, L = sample fermented with strain 5A,
- M = sample fermented with strain 6C, N = sample fermented with strain BC4333, O = Unfermented sample,
- P = commercial ‘iru-woro’, Q = commercial ‘iru-pete’.

Values are means of three replicate

Data are same with superscript in a column are not significantly different at P = 0.05

**Table 2: Proximate Composition of the Starter Culture-Fermented and Unfermented Samples of Parkia biglobosa**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Protein</th>
<th>Ash</th>
<th>Fat</th>
<th>Crude Fibre</th>
<th>Carbohydrate</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>37.4±0.00</td>
<td>3.58±0.20</td>
<td>20.8±0.49</td>
<td>7.37±0.76</td>
<td>26.73±0.68</td>
</tr>
<tr>
<td>B</td>
<td>34.40±0.92</td>
<td>2.48±0.31</td>
<td>25.72±0.49</td>
<td>6.88±0.12</td>
<td>26.25±1.29</td>
</tr>
<tr>
<td>C</td>
<td>33.43±0.25</td>
<td>3.48±0.17</td>
<td>25.11±0.35</td>
<td>8.11±0.36</td>
<td>23.98±0.81</td>
</tr>
<tr>
<td>D</td>
<td>33.32±0.35</td>
<td>3.55±0.24</td>
<td>23.70±0.37</td>
<td>7.13±0.10</td>
<td>22.32±1.20</td>
</tr>
<tr>
<td>E</td>
<td>42.61±0.01</td>
<td>3.12±0.02</td>
<td>26.98±0.38</td>
<td>7.83±0.25</td>
<td>14.96±1.20</td>
</tr>
</tbody>
</table>
The pH values of all the samples increased from 7.01 to 7.58 while that of the unfermented sample was nearly neutral or basic. The increase in pH could have encouraged the growth of Bacillus spp, which grows well at pH 7.0-8.0. The liberation of ammonia during fermentation of protein foods is a phenomenon observed by Odunfa (2004), during fermentation of ‘iru’. There was inverse correlation between the pH and the TTA. The decrease in acidity i.e. titratable acidity of the fermented samples may be due to hydrolysis of protein and abundant production of ammonia by various bio-chemical pathways. The moisture content of the samples which varied between 46.66% and 59.33% were closely related to that reported by Omafuvbe et al. (2004) who carried out similar research. The increase in the moisture contents of the samples may be due to hydrolysis of protein and abundant production of ammonia by various bio-chemical pathways. The moisture content of the samples which varied between 46.66% and 59.33% were closely related to that reported by Omafuvbe et al. (2004) who carried out similar research. The increase in moisture contents of the starter culture fermented ‘iru’ samples over the unfermented substrate might have been due to the addition of water during soaking, boiling and dehulling. It might have also been due to the activities of the micro-organisms on the ‘iru’ samples as a result of extracellular enzymes production. This result conforms to that of Omafuvbe et al. (2004) who carried out similar research on African locust bean and melon.

The ‘iru’ samples produced using Bacillus subtilis strains 3A and 2B were statistically rated better than commercial ‘iru-woro’, ‘iru’ produced using Bacillus subtilis strain BC4333 was rated better than commercial ‘iru-pete’, on all the parameters. The better performance of the starter cultures might be as a result of their extracellular enzymatic activities. Odunfa and Adewuyi, (1985b) reported that strains of Bacillus involved in fermentation of ‘iru’ could influence the quality of the product.

The increase in protein content of starter culture-fermented ‘iru’ the might be due to of the structural proteins that are integral part of the microbial cells (Tortora et al., 2002). The apparent increase in growth and microbial proliferation of microorganisms in form of single cell protein of the starter culture and normal flora may account for the observed trend in the crude protein (Oboh, 2006). The observed decrease in carbohydrate content of the starter culture-fermented products may be attributed to the leaching of the soluble carbohydrates like sugar into the cooking water and as a result of utilization

Table 2: Contd.,

<table>
<thead>
<tr>
<th></th>
<th>F</th>
<th>G</th>
<th>H</th>
<th>I</th>
<th>J</th>
<th>K</th>
<th>L</th>
<th>M</th>
<th>N</th>
<th>O</th>
<th>P</th>
<th>Q</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>33.47±0.32</td>
<td>43.44±0.16</td>
<td>36.68±0.24</td>
<td>33.70±0.20</td>
<td>34.50±0.30</td>
<td>33.79±0.96</td>
<td>36.01±0.65</td>
<td>35.74±0.17</td>
<td>39.40±0.09</td>
<td>31.26±0.32</td>
<td>40.51±0.06</td>
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<td>3.37±0.08</td>
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<td></td>
<td>25.01±0.42</td>
<td>28.86±0.22</td>
<td>24.10±0.44</td>
<td>26.25±0.49</td>
<td>7.37±0.76</td>
<td>24.27±0.19</td>
<td>25.27±0.64</td>
<td>25.03±0.40</td>
<td>26.25±0.49</td>
<td>20.93±0.43</td>
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<td></td>
<td>7.59±0.45</td>
<td>8.00±0.25</td>
<td>8.08±0.14</td>
<td>8.42±0.28</td>
<td>6.90±0.05</td>
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<td>7.30±0.19</td>
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<td></td>
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<td>18.60±0.53</td>
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<td>23.30±0.18</td>
<td>25.88±0.84</td>
<td>22.24±1.28</td>
<td>24.17±0.70</td>
<td>19.01±0.78</td>
<td>22.84±0.74</td>
<td>13.47±0.78</td>
<td>17.51±1.16</td>
</tr>
</tbody>
</table>

**Key:** A=sample fermented with strain 8B, B= sample fermented with strain1A, C = sample fermented with strain 9B, D= sample fermented with strain 10B, E = sample fermented with strain 2B, F= sample fermented with strain 7A, G= sample fermented with strain 3A, H= sample fermented with strain 4A, I= sample fermented with strain 6A, J= sample fermented with strain 3B, K= sample fermented with strain = 9A, L= sample fermented with strain 5A, M= sample fermented with strain 6C, N= sample fermented with strain BC4333, O=Unfermented sample, P=commercial ‘iru-woro’, Q-commercial ‘iru-pete’.

Values are means of three replicate

Data with same superscript in a column are not significantly different at P = 0.05

**DISCUSSIONS**

The increase in protein content of starter culture-fermented ‘iru’ the might be due to of the structural proteins that are integral part of the microbial cells (Tortora et al., 2002). The apparent increase in growth and microbial proliferation of microorganisms in form of single cell protein of the starter culture and normal flora may account for the observed trend in the crude protein (Oboh, 2006). The observed decrease in carbohydrate content of the starter culture-fermented products may be attributed to the leaching of the soluble carbohydrates like sugar into the cooking water and as a result of utilization
Effects of the Use of Starter Culture on the Quality of Fermented *Parkia biglobosa*

of some of the sugars by fermenting organisms for growth and metabolic activities. This result agrees with reports of earlier worker (Addy et al., 1995; Omafuvbe et al., 2004; Osman, 2007).

There was a significant reduction in crude fiber of the starter culture-fermented products as a result of fermentation. The reduction may be attributed to the fermenting microflora to hydrolyze and metabolize them as carbon source (substrate) in order to synthesize cell biomass. The ash content of the sample decreased significantly ($p = 0.05$) during fermentation, when compared with commercial ‘iru’ samples and the starter culture fermented ‘iru’. The loss in ash may be due to the utilization of these salts during fermentation by microorganisms for their metabolic activities. The increase in fat concentration as a result of fermentation may be attributed to boiling of the sample which might have led to the cleavage of the protein – lipid as carbohydrate – lipid linkages thereby, facilitating the easy extraction of the oil by the extracting solvent (Madigan, 2002).

REFERENCES


