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## Research Article Effect of Fermentation Time and Container on the Microbial and Sensory Properties of *Parkia biglobosa* Seed Condiment (Iru)

Popoola Olubunmi, Bolarin Funmilola Mary, Olotu Funke Bosede, Onyemize Uchenna Charles and Ademiluyi Yinka Segun

Department of Processing and Storage Engineering, National Centre for Agricultural Mechanization, P.M.B. 1525, Ilorin, Nigeria

### Abstract

**Background and Objective:** Iru is a traditional food condiment derived from solid-state fermentation of dried, processed seeds of African locust beans tree (*Parkia biglobosa*). Iru contribute protein, minerals and calories to the diet. The objective of this work was to determine the microbial and sensory properties of Iru fermented using different fermentation containers. **Materials and Methods:** Parkia seeds were processed and fermented for 72 hrs using different fermentation containers (stainless steel, plastic and calabash). The Iru produced was analyzed for microbial and sensory properties using standard methods. **Results:** The total microbial counts, coliform counts and sensory properties varied significantly (p<0.05) with values ranging thus: Total microbial counts ( $8.10 \times 10^4$ - $1.17 \times 10^5$  CFU), total coliform ( $0.50 \times 10^3$ - $7.20 \times 10^3$  CFU), appearance (3.00-6.20), taste (3.93-6.00), odour (3.43-6.10) and general acceptability (4.00-6.34), respectively. **Conclusion:** From this study, it can be concluded that fermentation in stainless steel offers the production of iru with acceptable sensory and microbial properties.

Key words: Fermentation, stainless, calabash, Iru, plastic, microbial

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**Corresponding Author:** Bolarin Funmilola Mary, Department of Processing and Storage Engineering, National Centre for Agricultural Mechanization, P.M.B. 1525, Ilorin, Nigeria

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

#### INTRODUCTION

*Parkia biglobosa* beans condiment, popularly known as Iru in the western part of Nigeria, is one of the most popular and widely accepted African food condiments and Iru and Ogiri are the two most popular indigenous fermented condiments produced from legumes and oil seeds<sup>1</sup>.

The acceptability of Locust bean Iru is based on the role it has played in the tradition of various cultures, it is used in soups by communities in several parts of the country<sup>2</sup>. Iru is a condiment produced from the fermentation of the dried, dehulled boiled seeds of the African locust beans tree-Parkia biglobosa, which is a perennial leguminous tree of the subfamily Mimosoideae family Leguminosae, it is a tree that is indigenous with economical and social importance to the African people. Iru production involves some very important unit operations the seeds are boiled on fire for about 24 hrs to soften the testa and cotyledons, the boiled, soft seeds are pressed in the mortar or with feet to remove the softened testa: after which the seeds are washed to remove the testa from the cotyledon and then boiled for another 1-2 hrs again. The fermentation of the seeds makes them edible by increasing their digestibility. Iru fermentation is known as a solid-state fermentation with an exothermic process, where the temperature of the fermenting seed increases gradually from ambient temperature to about 30-45°C<sup>1</sup>. The traditional fermented foods contain a high nutritive value, better digestibility and developed a diversity of flavours with improved nutritional guality and enhanced shelf stability and safety, aroma and texture in food substrate<sup>3</sup>. They have potential applications as supplements of protein, they also have applications as functional ingredients in food. In many traditional communities, soups are the main sources of protein and minerals and to improve the diet of rural dwellers improving the nutrient content of soups is a very innovative way.

Moreover, iru does not only contribute protein, minerals and calories in the diets it also serves seaning and flavouring agents in soups and sauces<sup>4</sup>. Legumes and oilseeds for the production of iru are fermented by allowing the microorganisms to act on the substrate, during which the microorganisms act on the components of the substrates, converting them into products that the nutritional composition and taste of the final product<sup>5</sup>. Aside from the reduction in anti-nutritional factors present in some oilseed and legumes, Fermentation has generally been observed to improve the nutritional qualities, flavour and safety of raw fermented food products<sup>6</sup>. However *Parkia* seed condiments are known to have significant value in the diet of many local regions, they are also known to be associated with short shelf life, unprotected and unacceptable packaging materials, characteristic odour as well as stickiness. All aforementioned have reduced their acceptability in some parts of the world. This work thus seeks to determine the microbial and sensory properties of Iru fermented using different fermentation containers and different fermentation times.

#### **MATERIALS AND METHODS**

**Study area:** The study was carried out at the food research laboratory of the National Centre for Agricultural Mechanization (NCAM), Ilorin, Kwara State.

**Materials:** African locust bean (*Parkia biglobosa*), fermentation containers (plastic bowls, stainless bowls and calabash) were obtained at Oja Oba Market in the llorin metropolis.

**Sample preparation:** The *Parkia biglobosa* seeds were sorted manually and washed to remove dirt, spoilt seeds and debris that were packed with the seeds during harvesting and processing 3.2 kg of seeds were boiled under pressure for 3 hrs to soften the cotyledons, this was followed by dehulling the cotyledons, using NCAM dehulling machine, then washed thoroughly in water. The washed seeds were then re-cooked for another 30 min and subsequently drained. The locust beans samples were later fermented for 24, 48 and 72 hrs in plastic, stainless steel and calabash in Fig. 1. Samples were then packed and dried under oven and sun drying methods.

#### Analyses

#### **Microbial analysis:**

**Total viable bacteria count:** One millilitre of the serially diluted mixture was aseptically poured into each sterile Petri dish containing nutrient agar (NA) using a sterile pipette. The inoculated plates were properly and gently mixed before being incubated at 37°C for 48 hrs. Colonies were counted with the counts done in three replicates using an electronic counter (FISON Model CNW-330-010X)<sup>7</sup>.

**Fungi count:** One millilitre of the serially diluted mixture was aseptically poured into sterile Petri plates containing potato dextrose agar using a sterile pipette. The plates were properly and gently mixed before being incubated at 30°C for 36 h. All counts were done in three replicates using an electronic counter (FISON Model CNW-330-010X)<sup>8</sup>.



Fig 1: Flow diagram for production and processing of Africa locust bean seeds to food condiments (Iru)<sup>4</sup>

**Sensory analysis:** Sensory evaluation was carried out using twenty panellists to assess the sensory attribute (colour, appearance, taste, odour, flavour and overall acceptability) of the food condiments (Iru). The panellists were selected randomly cutting across students and workers of NCAM which include people who are used to eating "Iru" and those who are not used to them. The samples were presented in coded identical plates. The panellists were instructed to rate the sample for the parameters based on a 9-point hedonic scale ranging from 9-liked extremely to 1-disliked. The raw scores were assembled and statistically analyze<sup>8</sup>.

**Statistical analysis:** Data obtained (triplicate) were subjected to Analysis of Variance (ANOVA) procedures using the statistical package for social sciences (SPSS) version 17 for the window. Mean separation was performed by the Duncan test ( $p\leq0.05$ ).

#### **RESULTS AND DISCUSSION**

**Effect of fermentation time and container on the microbial properties of Iru:** The effect of fermentation time and fermentation containers on the microbial properties of Iru is presented in Table 1. The bacteria count of fermented Parkia seeds varied significantly (p<0.05) ranging from  $8.10 \times 10^{4}$ - $1.17 \times 10^{5}$  CFU. During the first 24 hrs, samples fermented using plastic recorded the lowest count of bacteria, while most bacteria counts were recorded for samples fermented using calabash. CLBH had the highest TBC count  $(1.15 \times 10^5)$  CFU while PLST had the least value  $(9.75 \times 10^4)$ . At 72 hrs of fermentation, PLST had the highest TBC, while STLS and CLBH had  $7.50 \times 10^4$  and  $8.10 \times 10^4$  total bacterial count, respectively. Total coliform counts (TCC) also varied significantly ranged between  $1.50 \times 10^3$ - $7.20 \times 10^3$  CFU, during the first 24 hrs of fermentation, with the highest counts recorded for iru fermented using calabash. Values ranging from 19.67-34.67 CFU were recorded for total fungal count (TFC), CLBH at 48 hrs of fermentation having the highest value while CLBBH at 24 hrs had the lowest value. TCC and TFC were observed to decrease as fermentation times total bacterial counts, while TBC increase slightly.

Sensory evaluation of Iru fermented in different fermentation containers: The result of the sensory evaluation of Iru fermented using different fermentation containers is presented in Table 2. The appearance of Iru varied significantly and ranges between 3.00 and 6.20, which denotes dislike slightly to like moderately. However, it was observed that appearance preference decreases with increasing fermentation time.

The low bacteria count recorded in plastic and the most count recorded in calabash may explain the fact that stainless steel offers better protection against bacteria growth compared to calabash and plastic containers. However, it was observed that bacteria counts of Iru decreased with increasing fermentation time and higher counts were recorded during the early hours of fermentation lower counts were recorded towards the last hour of fermentation. This might be due to the decrease in the substrate as fermentation progresses. According to a previous study<sup>9</sup> worked on iru and sonru condiment reported that microbial counts were higher in both iru and sonru and increased sharply at the early stage of the fermentation.

The high fungal counts recorded for iru samples fermented using calabash might also be attributed to the possibility of contamination as a result of hygiene and reaction of products of fermentation and composition of the lining of the calabash. An increase in total coliform may be a result of faecal contamination from the water used during processing as well as a low level of hygiene during the handling of raw materials. This is in line with the findings of

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Table 1: microbial properties of Iru condiments fermented for 72 hrs

Samples	Total bacterial count	Total coliform count	Total fungi count 34.33±3.58ª	
STLS24	1.03×10 <sup>5</sup> ±2.12 <sup>9</sup>	4.25×10 <sup>3</sup> ±3.76 <sup>9</sup>		
PLST24	9.75×10⁴±5.01°	6.05×10 <sup>3</sup> ±3.53 <sup>a</sup>	24.67±2.37ª	
CLBH24	1.15×10⁵±1.67 <sup>h</sup>	7.20×10 <sup>3</sup> ±6.13 <sup>h</sup>	19.67±1.52ª	
STLS48	7.05×10 <sup>4</sup> ±1.69 <sup>a</sup>	3.35×10 <sup>3</sup> ±2.70 <sup>e</sup>	25.56±2.23ª	
PLST48	1.01×10 <sup>5</sup> ±1.37 <sup>f</sup>	3.60×10 <sup>3</sup> ±5.53 <sup>f</sup>	20.34±1.12ª	
CLBH48	8.20×10 <sup>4</sup> ±2.71 <sup>d</sup>	2.52×10 <sup>3</sup> ±5.23 <sup>g</sup>	34.67±3.09ª	
STLS72	7.50×10 <sup>4</sup> ±3.26 <sup>b</sup>	$1.90 \times 10^{3} \pm 2.54^{d}$	20.00±2.00ª	
PLST72	1.17×10 <sup>5</sup> ±1.24 <sup>i</sup>	1.70×10 <sup>3</sup> ±5.20 <sup>c</sup>	19.67±1.53ª	
CLBH72	8.10×10 <sup>4</sup> ±1.87 <sup>c</sup>	$1.50 \times 10^{3} \pm 1.00^{b}$	27.50±2.54ª	

#### Table 2: Sensory evaluation of Iru condiment

Samples	Appearance	Colour	Taste	Odour	Flavour	Overall acceptability
STLS24	5.97±1.16 <sup>de</sup>	6.20±110 <sup>de</sup>	5.41±1.59 <sup>b</sup>	4.97±1.67 <sup>bc</sup>	5.45±1.38 <sup>bcd</sup>	5.17±1.72℃
PLST24	5.03± 1.47 <sup>cd</sup>	5.50±1.23 <sup>cd</sup>	5.07±1.53 <sup>b</sup>	4.97±1.61 <sup>bc</sup>	5.14±1.48 <sup>bcd</sup>	5.17±1.54°
CLBH24	4.38±1.57 <sup>bc</sup>	4.90±1.56 <sup>bc</sup>	4.24±1.60ª	4.30±1.77 <sup>ab</sup>	4.55±1.53 <sup>ab</sup>	4.07±1.49ª
STLS48	6.20±0.96 <sup>e</sup>	6.43±0.82 <sup>e</sup>	6.00±1.31 <sup>b</sup>	6.10±1.45 <sup>d</sup>	5.97±1.30 <sup>d</sup>	6.17±1.18 <sup>e</sup>
PLST48	5.83±1.23 <sup>de</sup>	$5.93 \pm 1.048^{de}$	5.93±1.44 <sup>b</sup>	6.03±1.50 <sup>d</sup>	6.10±1.26 <sup>d</sup>	6.34±0.77 <sup>e</sup>
CLBH48	5.04±1.86 <sup>cd</sup>	4.90±1.94 <sup>bc</sup>	5.79±1.18 <sup>b</sup>	4.77±2.13 <sup>b</sup>	5.52±1.77 <sup>bcd</sup>	5.31±1.34 <sup>cd</sup>
STLS72	4.72±2.36 <sup>bc</sup>	5.00±2.19 <sup>bc</sup>	5.11±1.79 <sup>b</sup>	4.38±2.11 <sup>ab</sup>	4.86±2.10 <sup>abc</sup>	5.21±1.47°
PLST72	4.43±2.29 <sup>bc</sup>	4.53±2.08 <sup>abc</sup>	5.14±1.73 <sup>b</sup>	4.17±2.17 <sup>ab</sup>	4.86±2.29 <sup>abc</sup>	4.97±1.50 <sup>bc</sup>
CLBH72	3.00±1.97ª	3.87±1.87ª	3.93±1.59ª	3.43±1.91ª	3.89±2.03ª	4.00±1.53ª
CONTROL	3.97±2.60 <sup>b</sup>	4.07±2.20 <sup>abc</sup>	4.21±1.88ª	3.63±2.43ª	4.03±2.26ª	4.23±1.89 <sup>ab</sup>
	5.79±1.21 <sup>de</sup>	5.40±1.75 <sup>cd</sup>	5.79±1.35 <sup>b</sup>	5.83±1.29 <sup>cd</sup>	5.79±1.50 <sup>cd</sup>	6.00±1.02 <sup>de</sup>

Mean within the same column with different alphabets are significantly different (p $\leq$ 0.05)

the previous study<sup>10</sup>, that high-level total coliform is evidence of faecal contamination, which must have been acquired during handling and processing as well as evidence of poor unhygienic handling. It can be observed that only a slight decrease in the count of total bacteria was observed throughout the fermentation time. This may be attributed to the fact that bacteria are the predominant microorganism during the fermentation of iru and other condiments. According to other study<sup>8</sup> bacteria particular *Bacillus spp.* are the predominant microorganisms of African locust bean and castor oil seed condiments (Iru and Ogiri-isi) fermentation. Reduction in total fungal was also observed towards the last hour of fermentation, as well as total coliform. The presence of total coliform during the last hour of fermentation might still be attributed to faecal contamination during processing. However, the total viable count recorded in this study is in line with the total viable counts<sup>9</sup> on the fermentation of locust beans at 72 hrs. For fungal counts, it can be seen that no significant difference was observed.

Decrease in appearance preference might be due to the continuous degradation of substrate by microorganisms. This is in line with the result of other study<sup>8</sup>, who reported a similar trend in the production of parkia seed. However, the highest sensory score for taste was recorded during 48 hrs of fermentation in stainless steel container. A similar trend was

observed for colour, with Iru samples fermented in stainless steel during 24 and 48 hrs of fermentation been the most acceptable. The sensory score for taste varied significantly and ranged from 3.93-6.00. The sensory score for taste was observed to increase with increase in fermentation time. This might be due to the proteolytic activity of the fermenting bacteria on Parkia seeds that results into taste and flavor development as fermentation progresses<sup>1</sup>. Sensory score for odour varies significantly and ranges from 3.43-6.10, which denotes slightly dislike to like slightly like. The highest sensory score for odour was recorded iru samples recorded in stainless steel during 48 hrs of fermentation. The trend observed for flavor sensory score was guite different, as flavor scores increased during first 48 hrs of fermentation and decrease towards the last hour of fermentation. This might be due to the acids and flavour precursors produced during proteolytic breakdown of substrate by microorganisms in course of fermentation. However, iru sample fermented in plastic container was most acceptable for flavour with a sensory score of 6.10 which denotes like slightly.

The sensory score for general acceptability ranged from 4.00-6.34. It was however observed that only samples fermented in stainless steel and plastic container during 48 hours of fermentation were liked slightly while all samples irrespective of the fermentation time were either liked nor dislike, disliked slightly or disliked moderately.

#### CONCLUSION

Fermentation time and container were observed to influence the sensory and microbial properties of Iru. From this study, it can be concluded that fermentation in stainless steel offers the production of iru with acceptable sensory and microbial properties.

#### SIGNIFICANCE STATEMENT

This study revealed the effect of fermentation containers on the microbial and sensory quality of *Parkia biglobosa* (Iru) and this can be of great benefit in selecting the right fermentation container for processors and researchers. This study will help the researchers and processors to use the best fermentation container at the right fermentation time that gives the best iru quality. Thus, a new theory on the use of selected fomentation containers versus time may be arrived at and implemented.

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