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# Proximate Analysis and Sensory Evaluations of Iru Produced By *Staphylococcus* Sp. And *Bacillus* Sp. Separately

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***Abstract:*** *Three Staphylococcus sp. and three Bacillus sp. previously isolated from iru were used to ferment African locust bean, the pH and temperature of the fermenting mashes were monitored as fermentation progressed while the proximate analysis was carried out for unfermented bean, iru produced by Staphylococcus sp., iru produced with Bacillus sp. and iru produced with the mixture of the two bacterial groups. There was increase in the pH values of all of the fermenting mashes inoculated with different species of bacteria as fermentation hours increased. There was no significant difference in the odour, stickiness, colour, taste, mucilage and the general acceptability of the Staphylococcus sp. fermented iru, Bacillus sp. fermented iru, and the iru fermented with both Bacillus sp. and Staphylococcus sp. in combination. Staphylococcal fermented iru has the highest percentage carbohydrate compared with that fermented with Bacillus and that fermented with both bacteria species, and lower ether extract was observed for the Staphylococcal fermented iru when compared to that of Bacillus fermented iru.*

***Keywords****: African locust bean, Bacillus fermented iru, general acceptability, iru, Staphylococcal fermented iru.*

## I. Introduction

Iru (Dawadawa) is one of the most popular and widely accepted African food condiments, and the most popular food condiment in the region of West and Central African (7), the popularity and acceptance of Locust bean dawadawa was not in doubt as it is used in soups by communities in several parts of the country (14). Iru-a condiment produced from the fermentation of the dried, dehulled boiled seeds of African locust beans tree-*Parkia biglobosa*, which is a perennial leguminous tree of the sub-family *Mimosoideae* family *Leguminosae*, it is an indigenous tree with economical and social importance to the African people. Iru production involves the boiling of the dried seeds on fire for about 24h to soften the testa and cotyledons, the boiled, soft seeds are pressed in the mortar 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 hours again. The fermentation of the seeds makes them edible by increasing their digestibility (18). Iru fermentation is a solid substrate fermentation with an exothermic process, where the temperature of the fermenting seed increases gradually from ambient temperature to about $30^0$C to $45^0$C (18). Desired fermentation state is indicated by the fermentation state is indicated by the formation of mucilage and production of ammonia as a result of the breakdown of amino acids (22). Apart from the improvement of sensory properties, condiments add nutritional values to foods, providing dietary fiber, energy, minerals and vitamins (14). Iru is an important source of riboflavin and it contains the highest riboflavin content when compared to some other common plant foods (7,29).

The nutrition status of iru has the range (% dry basis) 31.2-47.4 crude protein; 3.3-6.8 ash, 31.20-42.9 fat and 3.6-23.6 carbohydrate by difference, (7,10). The protein though is not of high quality (7), the inherent meaty flavor of iru has made it a very important ingredient in many dishes in many parts of Africa. Odunfa (16); reported the product (iru) to be a moist solid with a variable moisture content of 43% in boiled unfermented product; he reported a gradual increase in temperature from $25^0$C in the fermenting mash which rose sharply after 24 hours, reaching a peak of $42^0$C and a gradually increased pH during the first 30 hour and later increased sharply to 8.1. Because of the fact that the major constituents of vegetable seeds are proteins, organisms responsible for their fermentation must be able to utilize their constituents (28).

Beaumont (6) suggested that the preservative and flavor characteristics of alkaline fermentations are derived partly from the liberation of ammonia and increase in pH. Ikenebomeh *et al.* (12) indicated that active microbial metabolism is required to bring about the changes observed in locust bean during fermentation. Omafuvbe *et al.* (23) reported an increase in pH of fermented soybeans after 72 hours of fermentation of which *Bacillus sp.* as single inocula showed different ability to increase the final pH value with different proteolytic enzyme activities and free amino acids production.

Ogunjobi *et al.* (20) reported a slight increase in the fat, protein and moisture contents of brined samples of Irish potatoes, but a reduction in the crude fiber, reducing sugar and carbohydrate contents. Azokpota *et al.* (5) that suggested that the increase in pH could be attributed to the abundant production of ammonia which characterizes proteinacious food fermentation; and that the increase in crude protein, fat, ash and crude fiber contents were as a result of a selective reduction in carbohydrate content due to the activities of the microorganisms which most likely derive their energy from carbohydrate metabolism.

This work thus seek to determine the changes in the proximate characteristics and the differences in the sensory characteristics of *Staphylococcus* produced iru compared with the iru produced with *Bacillus* and iru produced with the two bacteria species.

## II. Materials And Method

### 2.1 Fermentation of Iru in the Laboratory

Dried seeds of African locust beans were purchased from Bodija market in Ibadan and cooked in a pressure pot for about 2h and the swollen seeds were robbed in between palms to remove the testa, the hard seed were removed during washing. The cotyledons were then boiled for another 30 minutes and drained in a sieve. Fifty (50g) grams of boiled, dehulled seeds was weighed into small DANA plastics with air tight cover and sterilized at 121$^0$C for 15minutes, then allowed to cool down; 0.5 ml of inoculums was used to inoculate the cooked beans.

### 2.2 Inocula Preparation

Three *Staphylococcus* sp. (*S. saprophyticus, S. xylosus* and *S. epidermidis*) and three *Bacillus* sp. (*B. subtilis, B. licheniformis* and *B. pumilus*) previously isolated from iru (all gotten from the Food Laboratory of the Department of Microbiology of the University of Ibadan) were used. Each bacteria group was grown on Plate count agar at 37$^0$C for 18 h; each of the group was suspended in 10 ml sterile 1% NaCl solution, the suspensions were mixed equally for the mixed cultures and then diluted to give an absorbance of 0.03 at 540 nm in a UV-visible spectrophotometer, 0.5 ml of the suspension was used to inoculate the cooked beans (23; 25) acceptically (this gives about $10^4$ cells $g^{-1}$ wet wt.). The inoculated beans were incubated at 37$^0$C for 40 h. The experiments were carried out in triplicate and analysed at specific time.

### 2.3 Proximate analysis

Analysis of ether extract, moisture content, ash content and crude fiber of unfermented and fermented beans were carried out using AOAC (4) analytical method; crude protein was determined by Kjeldahl method while the crude carbohydrate was determined by difference.

### 2.4 pH and Temperature Measurement

The temperature and pH changes during fermentation were measured at 0, 12, 24, 36 and 48 h respectively. The pH was determined by mixing 1g of the fermenting mash with 10ml of distilled water and the suspension was used for the pH determination using a pH meter (using EXTECH pH100).

### 2.5 Sensory Evaluation

Organoleptic properties of the iru fermented with *Staphylococcus* sp., *Bacillus* sp.and the combination of the two were determined alongside with uncontrolled traditionally fermented iru following the method of Oner *et al.* (27) and Tamang and Nikkuni (30). The colour, odour, taste, mucilage and stickiness of samples were assessed by a set of 10 man panelist who are regular consumers of the iru, using a score range of 5 (like extremely) to 1 (dislike extremely) to grade their likeness for the oduor, taste and general acceptability; while a score range of 5 (strongly like iru) to 1 (not like iru at all) for colour, stickiness and mucilage.

### 2.6 Statistical Analysis

The data obtained for proximate and sensory evaluations were subjected to analysis of variance (ANOVA) and the Duncan's multiple range tests was used to separate the means while significant difference was obtained for sample treatments ($P \leq 0.05$).

## III. Results and Discussion

### 3.1 Changes in temperature

Table 1 shows the changes in temperature of the fermenting mash as fermentation progress. There was an increase in the temperature of all the fermenting mashes from 23.6$^0$C to a final temperature of 29.3$^0$C for the *Staphylococcus* sp. fermented locust beans and 23.7$^0$C to a final temperature of 28.9$^0$C for *Bacillus* sp. fermented beans while the one fermented with the two species of bacteria has a final temperature of 29.5$^0$C, from an initial temperature of 23.5$^0$C.

The increase in temperature during fermentation agrees with the report of Odunfa (16), who reported a sharp increase in the temperature of the fermenting marsh from 25$^0$C after 24h, the result is not at disparity with the result of Jonathan *et al*., (13) which showed a significant increase in the temperature of the fermenting mash from an initial temperature of $28^0$C to around $30^0$C at the 96h during the spontaneous fermentation of bambara nut to produce iru.

Table 1: Change in temperature ($^0$C) during African locust bean fermentation with different inocula for iru production

| Product | 0h | 12h | 24h | 36h | 48h |
|---|---|---|---|---|---|
| Staphylococcal iru | 23.6 | 26.2 | 26.8 | 27.7 | 29.3 |
| Bacillus iru | 23.7 | 26.4 | 26.8 | 27.1 | 28.9 |
| Bacillus + Staphylococcus Iru | 23.5 | 26.2 | 26.6 | 27.6 | 29.5 |

### 3.2 Changes in pH

The change in the pH of iru fermented with different inocula is shown in figure 1, the pH of the *Staphylococcus* sp. fermented locust beans increased from 6.46 at 0h to 7.59 at 12h and 24h, then declined to 7.55 at 36h, after which it rose again to 7.70 at 48h of fermentation. The pH of the *Bacillus* sp. fermented beans increased from 6.45 at 0h and 7.61 at 12 h of fermentation then declined to 7.56 and 7.55 at 24 and 36 h of fermentation respectively then increased to 7.65 after 48 h of fermentation. The pH of the locust beans fermented with the combination of the two species of organisms rose from 6.46 at 0h to 7.62 at 24h, then declined to 7.55 at 24h, then rose to 7.57 and 7.84 at 36h and 48h of fermentation respectively.

The fluctuations in pH of the fermenting mashes is not in agreement with the results of Odunfa (16) who reported an increase in pH from the beginning to the end of fermentation, but the results agree with his report of a sharp increase in the pH after 30h of fermentation. The pH at the start of fermentation and that after 48h of fermentation agrees with that of David and Aderibigbe (9) who reported the pH of the unfermented substrates ranged between 6.1-6.4 and 6.8-7.8 during fermentation, Omezuruike *et. al.* (26) whose result showed the pH level of 5.5 at the beginning of fermentation which increased to 8.0 at the end of fermentation, during the production of anyi, the result of Omafuvbe *et al.* (23), who reported an initial pH of 6.50-6.55 which increased to between 7.50-8.00 after 72 hours of fermentation also with that of Antai and Ibrahim (3) who reported a pH of 7.1at 0h, pH of 7.3 at 24h, pH 7.5 of at 48h and a pH 7.9 at 72h of fermentation but disagree with that of Jonathan *et al.* (13) who reported that the pH decreased from 6.7 at 0h to 4.4 at the end of fermentation of bambara nuts for the production of iru.

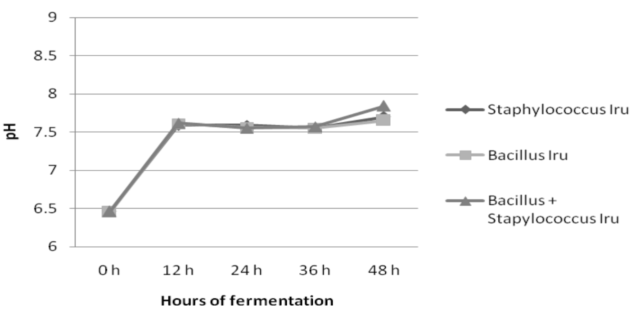


Figure 1: Change in pH of fermenting mash during the production of iru with different inocula

### 3.3 Sensory evaluation result

The result of the analysis of variance (ANOVA) for the sensory tests is shown in Table 2, there was no significant difference in the odour, stickiness, colour, taste, mucilage and the general acceptability of the *Staphylococcus* sp. fermented iru, *Bacillus* sp. fermented iru, and the iru fermented with both *Bacillus* sp. and *Staphylococcus* sp. when compared; this agrees with the work of Omafuvbe (25) who reported that there were no significant differences in the colour, aroma, taste and general acceptability of salt-free daddawa and 1% salted daddawa, also Jonathan *et al.* (13) noted that there was no significant differences in the colour, texture and the general acceptability of the iru produced from bambara nut to that produced from African locust bean, it is also in line with that of Aderibigbe and Odunfa (1) who suggested that though high lipase activity can lead to rancidity in fermented foods, it may also lead to the production of some volatile fatty acids, providing flavor and aroma since *Staphylococcus* sp. have been reported to produce lipase(19,15).

Table 2: Sensory Evaluation of Iru after 48h Fermentation with Different Inocula

| Organism | Colour | Stickiness | Mucilage | Taste | Odour | General Acceptability |
|---|---|---|---|---|---|---|
| *Staphylococcal* iru | $4.33^{a}$ | $3.67^{a}$ | $4.00^{a}$ | $4.50^{a}$ | $4.67^{a}$ | $4.50^{a}$ |
| *Bacillus* iru | $4.50^{a}$ | $4.00^{a}$ | $4.00^{a}$ | $4.33^{a}$ | $4.17^{a}$ | $4.17^{a}$ |
| *Bacillus+Staphylococcal* Iru | $4.50^{a}$ | $4.50^{a}$ | $4.50^{a}$ | $4.50^{a}$ | $4.17^{a}$ | $4.50^{a}$ |

a. Mean values followed by the same superscript are not significantly different by Duncan's Multiple Range test ($P \leq 0.05$)
b. **Key:**

5= like extremely, 4= like, 3= indifferent, 2= hate, 1= hate extremely (for taste, odour and general acceptability)
5= strongly like iru, 4= like iru, 3= not sure, 2= not like iru, 1= not like iru at all (for colour, stickiness and mucilage)

### 3.4 Result of the proximate analysis

Result of the proximate analysis of the iru fermented with different inocula is shown in Table 3; *Staphylococcal* fermented iru has the highest percentage carbohydrate compared with that fermented with *Bacillus* and that fermented with both bacteria species, and lower ether extract was observed for the *Staphylococcal* fermented iru when compared to that of *Bacillus* fermented iru. There is no significant difference in the ash content of the three fermented iru products, with an increase in their ash content compared to that of the unfermented beans. There was a significant difference in the protein content of the three fermented iru products, with a significant difference between the unfermented and the *Staphylococcal* fermented iru. There is no significant difference observed in the crude fiber content of the *Staphylococcal* iru and *Bacillus* iru.

Decrease in the carbohydrate content of iru after fermentation may be as a result of the ability of the microorganisms to utilize it as their major carbon source during fermentation and this is in agreement with the works of Jonathan *et al.* (13), Omafuvbe *et al.*, (24), Azokpota *et al.* (5) and that of Ogunjobi *et al.* (20), who individually reported a decrease in the percentage carbohydrate contents of different protenaceous beans after fermentation to produce different condiments; the reduction in carbohydrate content may as well be due to the hydrolytic effect of microbial amylase converting carbohydrate into sugars.

There was a significant difference in the increased crude protein after fermentation of African beans with different inocula, with the one fermented with both *Bacillus* sp. and *Staphylococcus* sp. having the highest crude protein (42.10), followed the *Bacillus* sp. fermented iru (40.25) and then the *Staphylococcus* sp. fermented iru (39.90), this may be due to the ability of *Bacillus* sp. to carry out proteolysis more than *Staphylococcus* sp., but the two have a complimentary ability in producing the enzyme during the fermentation of African locust bean to produce iru. This increase in the crude protein of the products is in agreement with the works of Pelig-Ba (29), Dakwa *et al.*, (8), Jonathan *et al.* (13), Azokpota *et al.* (5), Antai and Ibrahim (3), Ogunjobi *et al.* (20), and Omafuvbe *et al.*, (24).

Also the change in crude protein, ash, ether extract, crude fiber and carbohydrate contents of the African locust beans after fermentation may be as a result of selective reduction in carbohydrate content due to the activities of the microorganisms which most likely derive their energy from carbohydrate metabolism (5).

The significant difference and increase observed in the moisture content of African locust beans after fermentation is in line with the report of Antai and Ibrahim (3), Ogunjobi *et al.* (20) and Jonathan *et al.* (13), who reported an increase in the moisture content of bambara nuts after fermentation; but this result differs from the report of Omafuvbe *et al.*, (24), who reported a decrease in the moisture content of African locust beans after 72h of fermentation.

Table 3: Proximate Analysis of Iru fermented with different inocula

| Sample | Moisture Content | % Crude Protein | % Ash | % Ether Extract | % Crude Fiber | % Carbohydrate |
|---|---|---|---|---|---|---|
| Unfermented beans | $47.73^{a}$ | $37.80^{a}$ | $2.00^{a}$ | $36.00^{a}$ | $4.00^{a}$ | $19.70^{c}$ |
| Staphyloccocal Fermented iru | $50.33^{b}$ | $39.90^{b}$ | $2.00^{a}$ | $35.50^{a}$ | $4.50^{a}$ | $18.10^{b}$ |
| Bacillus Fermented iru | $51.67^{c}$ | $40.25^{c}$ | $2.00^{a}$ | $36.00^{a}$ | $4.20^{a}$ | $17.65^{a}$ |
| Bacillus + Staphyloccocal Iru | $57.60^{d}$ | $42.10^{d}$ | $1.50^{a}$ | $35.00^{a}$ | $4.50^{a}$ | $17.40^{a}$ |

a. Mean values followed by the same superscript are not significantly different by Duncan's Multiple Range test ($P \leq 0.05$)

## IV. Conclusion

The result of the sensory test shows that the iru produced by *Staphylococcus* sp. is acceptable to the consumers, as the proximate analysis shows improved characteristics of the iru produced with both *Staphylococcus* sp. and *Bacillus* sp., this proves the ability of *Staphylococcus* sp. to aid the work of *Bacillus* sp. in the fermentation of African locust bean to produce iru.

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