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Microbiological Evaluation of 'Iru' and 'Ogiri-Isi' Used As Food Condiments

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Abstract: *Food condiments 'Iru powder' and 'Ogiri-isi' were produced from African locust bean (Parkia biglobosa) and Castor oil seed (Ricinus communis) respectively,using traditional method. Raw samples were dehulled and fermented for 96hours, dried and packaged.The organisms associated with the fermented products were identified as Bacillus subtilis, Enterococcus faecalis, Staphylococcus aureus, Saccharomyces cerevisiae, Penicillium spp, Rhizopus stolonifer and Saccharomyces cerevisiae var ellipsoideus. The pH changes occurring during the fermentation of the seeds were monitored. The pH increased proportionately with the fermentation period, ranging from 6.31 to 7.20 in African locust bean and 6.36 to 7.15 in Castor seed within the 96hours. The total microbial counts of "Iru" on Nutrient Agar (NA) and Potato Dextrose Agar (PDA) were 5.50 × 10^9cfu/g and 2.45 × 10^{10}cfu/g respectively while the total microbial counts of "Ogiri-isi" on NA and PDA were 3.07 × 10^{12}cfu/g and 2.41 × 10^{10}cfu/grespectively.*

Keywords: *Castor, fermentation, 'iru', microorganisms 'ogiri-isi', locust bean.*

I. Introduction

'Iru' and 'ogiri are the two most popular indigenous fermented condiments produced from legumes and oil seed [1]. 'Iru' is the Yoruba name for the fermented condiment produced from African locust bean (*Parkia biglobosa*)[2]. It is also known as 'dawadawa' in hausaland and by different names among ethnic groups [3]. 'Ogiri' is the name used by igbos for the traditionally prepared fermented condiments based on vegetable proteins. It is obtained by fermenting melon seeds (*Citrullus vulgaris*), fluted pumpkin (*Telferia occidentallis*) and castor oil seeds (*Ricimus communis*) [4].These raw materials are used to create the different varieties of 'ogiri' such 'ogiri-egusi,' 'ogiri-ugu', 'ogiri-isi' and 'ogiri-okpiye'[5].

The bulk of the indigenous fermented condiments of Nigeria are found in the southern states of Nigeria. Interstate trade and relocation has however, widened the scope of the spread throughout the country and beyond [6]. 'Iru' and 'Ogiri' have played major roles in the food habits of communities in the rural regions serving not only as a nutritious non-meat protein substitute but also as condiments and flavouring agents in soups and sauces [5]. They have potential good uses as protein supplement and as a functional ingredient. Soups are the main sources of protein and minerals and one of the ways to improve the diet is to improve the nutrient content of soups, According to [7], the traditional fermented foods contain high nutritive value, better digestibility and developed a diversity of flavours, aroma and texture in food substrates.

In addition 'iru' and 'ogiri' contribute protein, minerals and calories in the diets [8]. Legumes and oil seeds are fermented by allowing the microorganisms to act on them through enzymatic activity to yield condiments by the extensive hydrolysis of carbohydrate and protein components [9] and [10]. Apart from reduction in the anti-nutritional factors, fermentation markedly improved the digestibility, nutritive value and flavours of the raw seeds [11] and [12].Although 'iru' and 'ogiri' condiments constituted significant proportion of the diet of many people, they are associated with some problems such as having a short shelf life, objectionable packaging material, the characteristic putrid odour and stickiness[13].

The production of condiments is largely on a traditional small-scale, household basis under highly variable conditions [14]. In addition, the fermentation is usually carried out in a moist solid state, involving contact with appropriate inoculum of assorted microorganisms and is accomplished by the natural temperatures of the tropics.

II. Materials And Methods

2.1 Sample Collection and Preparation

The African locust bean (*Parkia biglobosa*) and castor oil seed (*Ricinus communis*) used in this study were brought from a local market at Nsukka, Enugu state and Ngodo, Umunneochi L.G.A, Abia state respectively. The 'iru' and 'ogiri-isi' were produced in the laboratory of Department of Food Science and Technology, Federal University of Technology, Owerri and Dr. Wesley Braide Laboratory, Nekede,owerri as outlined in Fig. 1 and 2 below.

2.1.1 Production of 'Iru' using Traditional Method

Raw African locust bean was boiled for 12h to soften the firmly attached seed coasts and further soaked in the boiling water for another 12h. Excess water was drained off and the seeds were dehulled by slightly pounding the seeds with a large wooden mortar and pestle and further removal of the seed coat was achieved by rubbing the cotyledons between the palms of the hand and washing with water. The cotyledons were again cooked for another 6h, the hot boil water was drained off and the cotyledons were then spread into calabash trays, covered with wooden trays, wrapped with juts sacks to keep the system warm and fermented for 4days to produce 'iru'. The 'iru' was then dried, ground and sieved to produce 'iru' powder.

2.1.2 Production of 'Ogiri-isi' using Traditional Method

Castor oil seeds were dehulled and then sorted to remove bad seeds and unwanted materials. The cotyledons were wrapped in blanched banana leaves and boiled for 8h to soften. Then, it was left to ferment at the prevailing ambient temperature (32-35^{0}C) for 4 days. At the end of the fermentation period, the seeds were ground into a paste and paste was wrapped up again into 'ububa' leaves and left near the fire place for one more day, when the unique aroma of 'ogiri-isi' was expected to have developed and then dried in the oven.However, the samples were kept in a cellophane bags, and stored in a refrigerator at 4^{o}C until required for analysis.

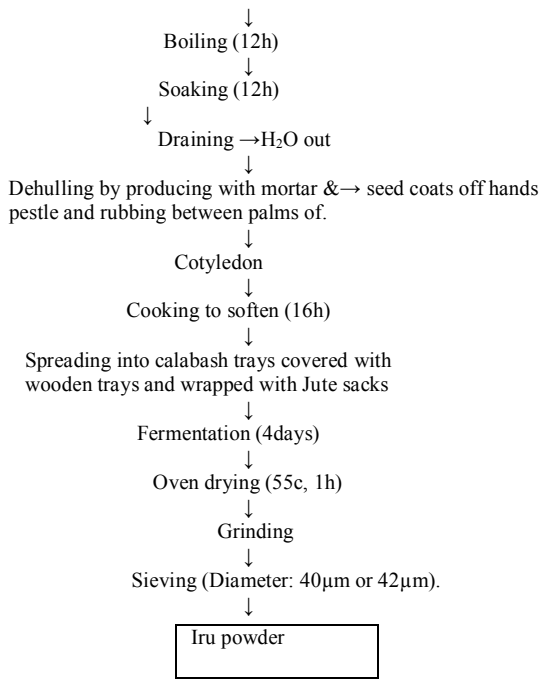
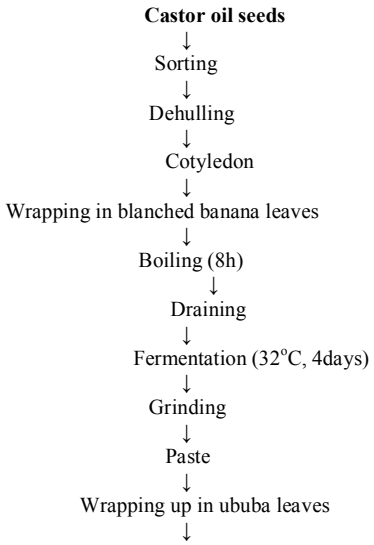


Figure 1: Flow chart for the production of Iru powder



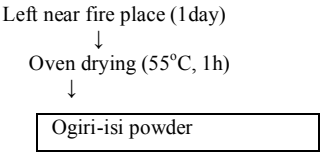


Figure 2: Flow chart for the production of Ogiri-isi

2.2 Microbiological Analysis

One gram of sample was diluted serially in ten folds dilution blanks and properly mixed with sterile glass rod [15]. The 0.1ml of diluted sample was introduced into sterile plate and molten sterile agar medium (45°C) was poured [16]. The media used were Nutrient Agar (NA), Potato Dextrose Agar (PDA) and Peptone Water Broth (PWB). The plates were rotated gently to disperse the inoculum in medium and allowed to solidify. Then the plates were incubated at 37°C.

2.3 Characterization of Isolates

Colonies that developed on the plates were grouped on the bases of their cultural characteristics. Pure cultures of all bacterial isolates were obtained by repeated streaking on NA, PDA and PWD plates. Morphological characteristics of each isolate were examined after Gram-staining and Motility under light microscope (1000) using oil immersion objectives for the purpose of identification, the following biochemical tests were performed on the isolates: catalase, indole, coagulase, citrate ,oxidase and sugar utilization (Glucose ,maltose, lactose, sucrose and mannitol).

2.4 Determination of pH

A wrap of the fermenting seeds was taken at the start of fermentation and at 24h interval for 4 days. Five grams (5g) of each samples was weighed into a sterile mortar and mashed with clean beaker and 50ml of distilled water was added. It was mixed thoroughly to form slurry. A standard buffer solution (pH 6.0) was prepared and this was used to standardize the pH meter (Dye Unicam,Model PW 9409). The electrode of the digital pH meter was dipped in the slurry. The pH readings were recorded.

III. Results

Table 1: Mean pH values of Fermenting Seed at different Fermentation Periods.

Periods of fermentation (hours)	'iru' from African locust bean	'Ogiri' from Cator oil seed
0	6.31	6.36
24	6.38	6.37
48	6.48	6.42
72	6.76	6.81
96	7.20	7.15

Table 2: Total Microbial Counts (cfu/g) of Raw and Fermented African Locust Bean Oil Seed on two Culture Media

Samples	Nutrient agar (10^9)	Potato dextrose agar (10^7)
Raw ALB	TNTC	1.7×10^7
Iru	5.50×10^9	2.45×10^{10}
Raw COS	TNTC	1.4×10^7
'Ogiri-isi'	3.07×10^{12}	2.41×10^{10}

Key:
TNTC = Too numerous to count
ALB = African locust bean
COS = Castor oil seed

Table 3: Total Count and Colonial Characteristics of Fungi on Potato Dextrose Agar

Sample Code	Total count (cfu/g)	Colony code	Colonial characteristic	Microscopic appearance	Probable Identity of isolates
A	1.7×10^7	PA_1	Large cream circular butyrous and mucoid colonies	Gram positive ellipsoidal and oval budding cell	*Saccharomyces cerevisiae var elliopsoideus*
B	1.4×10^7	PB_1	Dark green rough surface visible mass without visible spores	Irregular branches conidiophores, small conidia seen borne on larger ones	*Penicillium sp*
C	2.45×10^{10}	PC_1	Tiny cream circular colonies	Gram positive spherical budding	*Saccharomyces cerevisiae*

				cells	
		PC_2	Large cream mucoid and butyrous colonies	Gram positive ellipsoidal oval budding cells	*Saccharomyces cerevisiae*
		PC_3	White filamentous like hyphae	Non-septate hyphae spores	*Rhizopus stolonifer*
D	2.41 x 10^{10}	PD_1	Tiny cream circular colonies	Gram positive ellipsoidal budding Cells colonies	*Saccharomyces cerevisiae*
		PD_1	Large cream mucoid and butyrous colonies	Gram positive ellipsoidal oval budding cells	*Saccharomyces cerevisiae var ellipsoideus*

Key:
Sample A: Raw African locust bean
Sample B: Raw castor oil seed
Sample C: Fermented African locust bean (iru)
Sample D: Fermented castor oil seed, (ogiri-isi)

Table 4: Total Count and Colonial Characteristics of Bacteria on Nutrient Agar.

Sample code	Total count (cfu/g)	Colony Code	Size (mm)	Shape	Elevation	Colour	Margin	Surface appearance
A	TNTC	NA_1	4-6	IR	Flat	Cream	SR	D/D
B	TNTC	NB_1	1	R	LC	Cream	lint	M/S
		NB_2	1-2	IR	Flat	Cream	SR	D/D
C	5.50 xlO^9	NC_1	1-2	R	LC	Cream	lint	M/S
D	3.07 xlO^{12}	ND_1	5-8	IR	Flat	Cream	SR	D/D
		ND_2	1-2	R	LC	Cream	Ent	M/S

Key:
TNTC, Too numerous to count at 10^9 dilution; R, round; LC, Low convex; Ent, entire; M/S, Moist and shiny; D/D, dull and dry; IR, irregular; SR,
Serrated; Elev. Elevation
Sample A: Raw African locust bean
Sample B: Raw castor oil seed
Sample C: Fermented African locust bean (iru)
Sample D: Fermented castor oil seed, (ogiri-isi)

Table 5: Morphological and Biochemical Characteristics of Bacterial isolates on Nutrient Agar

Colony code	Microscopic characteristics	Cat	Oxi.	Coag.	In	Cit	Mot.	Sugar fermentation G	Mn	L	S	M	Most probable identity
NA_1	+ R beaded	+	-	-	-	+	+	+	+	+	+	+	Bacillus sp
NB_1	+ S chain	+	-	-	-	+	-	+	+	+	-	+	Enterococcus faecalis
NB_2	+ R beaded	+	-	-	-	+	+	+	+	+	+	+	Bacillus sp
NC_1	+ R short chain	+	-	-	-	+	+	+	+	+	+	+	Bacillus sp
ND_2	- R single	+	-	+	-	+	-	+	+	+	+	+	Staphylococcus aureus

Key:
Cat, catalase; oxi, oxidase; coag, coagulase; in, indole; cit, citrate; mot, motility;
G, glucose; L, lactose; S, sucrose; M, maltose; Mn, mannitol; R, rod shaped; S, spherical shape
Sample A: Raw African locust bean
Sample B: Raw castor oil seed
Sample C: Fermented African locust bean (iru)
Sample D: Fermented castor oil seed (ogiri-isi)

IV. Discussion

Table 1 shows mean pH values of fermenting seeds of different fermentation periods. The pH values of 'iru' and 'ogiri' before fermentation were 6.31 and 6.36 respectively, after 96h of fermentation, the pH of 'iru' and 'ogiri' was 7.20 and 7.15 respectively. The progressive increase in pH of fermented legumes and oil seeds compounds to other materials under similar conditions have been attributed to higher protein contents of these seeds [17] and [18].

[19] reported that during the fermentation of 'egusi', the total of unsaturated fatty acids increased with hydrolysis of protein into amino acids and peptides. Ammonia is released due to the proteolytic activity taking place during fermentation which therefore, raises the pH of the final products and giving the food a strong ammonical odour and flavor. [20] referred such fermentation as "alkaline fermentation" and this aids in prolonging shelf life of such products.

On the basis of morphological, cultural and biochemical characteristics, a total of eight microorganisms were identified and includes four bacterial and four fungal isolates. The bacterial isolates were identified as *Bacillus subtilis*, *Enterococcus faecalis*, *Staphylococcus aureus* and *Bacillus sp* as shown in Table. 5 while fungal isolates were identified as Saccharomyces cerevisiae, *Penicillium sp*, *Rhizopus stolonifer* and *Saccharomyces cerevisiae var ellipsoideus*; as shown in Table 3. [4] had reported that members of *Bacillus sp*, *Staphylococcus sp*, *Rhizopus* and *Penicillium sp* are the microorganisms involved in the production of 'Iru'. However, most researchers had also reported that *Bacillus* and *Staphylococus sp* as the predominant bacteria involved in the fermentations [21]; [22]; [23].The total microbial load of 'Iru' on Nutrient Agar plate and Potato Dextrose Agar plates were $5.50 \times 10^{9}$and 2.45×10^{10} respectively while the total microbial load of 'Ogiri-isi' on Nutrient Agar plate and Potato Dextrose Agar plates were $3.07 \times 10^{12}$and 2.41×10^{10} respectively (Table 2).

However, [24] reported 83% to 93% of the total isolates in 'Iru' to be *Bacillus sp*, while other organisms constituted 7% to 17% of the isolates. Also, [25] isolated a percentage of 19.4% *Bacillus sp* from 'Ogiri' samples obtained from different sources. This shows that *Bacillus sp* is the predominant microorganism in the fermentation of African locust bean and castor oil seed 'Iru' and 'Ogiri-isi'. From the health point of view, the presence and isolation of pathogenic organisms such as *Staphylococcus aureus*, *Enterococcus feacalis* and some *Bacillus sp* such as *Bacillus cereus* indicated poor hygienic practices during production, and have the potential to produce diarrheal toxin [26]; [27]. Although 'ogiri' and 'iru' has not been implicated in any form of mycotoxicity unlike fermented foods of South East Asia that were fermented mainly by moulds [28]; a practice of consuming 'iru' and 'ogiri' that has not been subjected to heat treatment should be discharged.

V. Conclusion

This research work revealed that *Bacillus subtilis* is the predominant microorganism involved in the fermentation of African locust bean and castor oil seed to 'iru' and 'ogiri-isi' respectively as food condiments. The work has also indicated the possibility of up-grading 'iru' and 'ogiri-isi' production to cottage industry by using the predominant microorganism as starter culture and also, by standardizing the processing conditions for the fermentation i.e. duration, temperature and methods of aeration during fermentation of the substrate. However, the ease of production is more with castor oil seed (*Ricinus comminus*) than the African locust bean (*Parkia biglobosa*) which is commonly available as the castor oil seed. Moreso, the dehulling of African locust bean is difficult and need to be mechanized.

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