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Major Fermentative Organisms in Some Nigerian Soup Condiments

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Abstract: Various types of microorganisms were isolated from fermented products of locust bean seeds (*Parkia biglobosa*), castor bean seeds (*Ricinus communis*), African oil bean seeds (*Pentaclethra macrophylla*) and mesquite seeds (*Prosopis africana*) and characterized. The fermented products, namely, iru, ogiri, ugba and okpei, respectively, are mainly used as condiments in soups, sauces and porridges among consuming populations in Nigeria. The results show that only bacteria were isolated from the fermented condiments. The organisms isolated included species of *Micrococcus*, *Lactobacillus*, *Staphylococcus* and *Bacillus*. From the results of morphological and biochemical tests carried out on the isolated species, *Bacillus subtilis*, *Bacillus cereus*, *Lactobacillus brevis*, *Lactobacillus fermenti*, *Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Micrococcus roseus* and *Micrococcus varians* were found to be present. The isolates were used to ferment freshly prepared oilseed samples, with subsequent evaluation of the desirable quality characteristics of texture, color and aroma. *B. subtilis* was found to give the products with acceptable quality attributes.

Key words: Soup condiments, fermentative organisms, locust bean seeds, castor bean seeds

Introduction

The prevailing population pressure in Nigeria, as in other less-developed countries, has resulted in an increasing demand for wild under-exploited nutritious plant products with aesthetic and organoleptic appeal in the daily diet (Enujiugha, 2005). The common edible portions of most under-utilized plants are the seeds, which in some cases are cooked or roasted and eaten directly as snack foods e.g. conophor nut and bambara groundnut, while some are cooked and fermented for use as soup and sauce ingredients e.g. African oil bean, locust bean, castor bean and melon. There are various plant seeds that are fermented and used as food in some rural parts of Nigeria, among which are, 'iru' from African locust bean (*Parkia biglobosa*), 'ogiri' from castor bean (*Ricinus communis*), 'okpei' from mesquite seed (*Prosopis africana*) and 'ugba' from African oil bean (*Pentaclethra macrophylla*). Diverse groups of bacteria comprising species of *Bacillus*, *Micrococcus*, *Leuconostic*, *Staphylococcus* and *Enterobacteriaceae* have been reported by various authors (Enujiugha and Badejo, 2002; Anosike and Egwuatu, 1981; Obeta and Ugwuanyi, 1996; Odunfa, 1981) as contributing to the individual fermentations. However, the indigenous fermentations are mostly achieved via natural inoculations. The need to standardize the processing techniques and to obtain hygienic and safe products, has led to the search for microbial starters for these local fermentations. The objective of the present study was to characterize the fermentative organisms with a view to identifying the major starters.

Materials and Methods

Materials: Traditionally fermented products of African oil bean (ugba), locust bean (iru), mesquite seed (okpei) and castor bean (ogiri) were bought from the King's market at Akure, Nigeria. Locust bean seed, African oil bean seed, mesquite seed and castor bean seed were obtained from local farmers at Owena in Ondo State, Nigeria. Aluminum foil used for the work was bought from the King's market. The syringes and cotton wool were purchased from Matador Pharmaceutical in Akure. Ethanol and simple sugars used in the study were bought from Wintech chemicals at Akure. All the chemicals and reagents used in the study were of analytical grade.

Isolation of microorganisms: Fermented samples (ugba, ogiri, iru, okpei) were taken aseptically from traditionally fermented beans. One gram of the sample was thoroughly mashed with laboratory pestle and mortar and mixed with 9 ml of normal saline water as a diluent in a McCartney bottle and the content was thoroughly shaken. Subsequent serial dilutions (10^{-2} , 10^{-3} , 10^{-4} , 10^{-5} , 10^{-6}) were made from this solution by adding serially 1ml of solution from preceding concentration to 9ml of the diluent, using sterile syringe. The nutrient agar was prepared by adding 11ml of distilled water to 2.8 g of agar in a conical flask. Unto a petri-dish containing 0.1 ml of the inoculum, 10 to 15 ml of the sterile warm (45°C) nutrient agar was poured. The plates were labeled appropriately, inverted and incubated at 30°C for 24 hrs in a Gallenkamp incubator.

Characterization and identification of isolates:

Colonies obtained after incubation were sub-cultured on nutrient agar which was incubated for 24 hours at 30°C. The cultural characteristics of isolates on the agar plates were observed. The motility of the isolates was examined using hanging drop technique. Gram staining reactions and cell morphology from heat fixed smears were done. The identification procedures for the microorganisms were carried out using Cowan and Steel (1966) methods. Pure cultures of the different organisms isolated were sub-cultured and preserved on agar slants at refrigeration temperature (4°C).

Laboratory fermentation of ugba, okpei, ogiri and iru with identified isolates:

The fermentation was carried out as shown on Fig. 1. The seeds of *Parkia biglobosa*, *Prosopis africana* and *Pentaclethra macrophylla* were boiled to soften the hulls for easy removal and separation of the cotyledons. *Ricinus communis* seeds were mechanically dehulled without parboiling. African oil bean seeds were sliced using a kitchen knife into a thickness of about 0.2-0.4cm. The dehulled and sliced beans were washed several times with clean water and boiled for 2-3 hours. The boiling serves to further soften the seeds and remove some of the anti-nutritional substances. The dehulled and boiled seeds were washed again with clean water and then soaked for 12 hours in tap water. After the soaking period, the seeds were drained and washed again until they were thoroughly cleaned. Mashing was done for castor bean seed only. The seeds were packed in washed banana leaves, about 30g in each wrap. The wraps were packed inside a container and sterilized in the autoclave at 121°C for 15 mins. 10 ml of Nutrient both were poured into test tubes and sterilized at 121°C for 15 mins. They were allowed to cool down to 25°C, a loopful of the organisms from the agar slants were transferred into the broth and incubated at 37°C for 24 hrs. The cell suspensions obtained were shaken together and 1ml was taken using a sterile syringe for the different organisms obtained from the traditionally fermented seeds. The wraps were allowed to ferment for 4 days in an incubator. Each wrap fermented with a different organism was evaluated for fermentative ability in bringing about desirable changes in color, texture, aroma and overall acceptability of the fermented beans seeds.

Sensory analysis: A panel of 10 adults who were conversant with the quality parameters evaluated the products. The panel rated the products based on color, texture and aroma. The rating was on 7 point hedonic scale where: 7 was dislike extremely, 4 was intermediate and 1 was like extremely for aroma and color. For texture, 7 was extremely hard, 4 was intermediate and 1 was extremely soft. Data obtained for

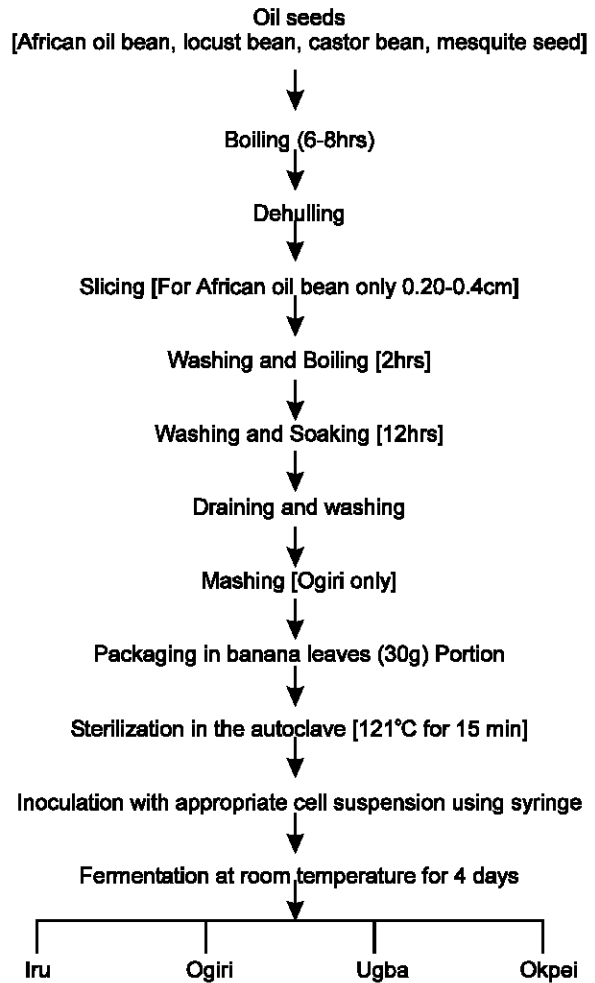


Fig. 1: Flow chart for the fermentation of Iru, Ogiri, Ugba and Okpei

all parameters were reported as means of 10 judgments. Analysis of variance (ANOVA) was computed for each sensory attribute (Snedecor and Cochran, 1976). Differences among sample means were separated using least significant difference (LSD) test.

Results and Discussion

Microorganisms isolated from the fermented iru, ogiri, ugba and okpei: Since the major constituents of these seeds are proteins, fats and carbohydrates, the organisms responsible for fermenting them must be capable of utilizing these three constituents. Most of the organisms isolated from the fermented seeds are known to possess such characteristics. The organisms isolated from fermented iru were *Bacillus*, *Micrococcus* and *Staphylococcus* species. *Bacillus* species were the predominant microorganisms present. These are known to have proteolytic ability and are also able to break down oils (Forgarty *et al.*, 1974). *Bacillus subtilis*

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Table 1: Colony Characteristics of the Isolates

Isolates	Colony Morphology on Agar Cell	Characterization	Probable Identification
OK1	Cream, circular, opaque, flat, rough	Gram +ve, long rod, central spore	<i>Bacillus sp</i>
OK2	Cream, circular, opaque, lobate, rough	Gram +ve, long rod in chain	<i>Bacillus sp</i>
OK3	Cream, circular, smooth, entire	Gram +ve rods	<i>Lactobacillus sp</i>
OG1	Cream, opaque, raised, lobate, smooth	Gram +ve long rod in cluster and singly	<i>Lactobacillus sp</i>
OG2	Cream, circular, opaque, rough and flat	Gram +ve long rod with central spore	<i>Bacillus sp</i>
OG3	Yellow, rhizoidal, opaque, rough and raised	Gram +ve cocci singly	<i>Micrococcus sp</i>
UG1	Cream, circular, opaque, flat, rough, entire	Gram +ve long rod with central spore	<i>Bacillus sp</i>
UG2	Cream, circular, raised and smooth	Gram -ve cocci, singly	<i>Micrococcus sp</i>
IR1	Irregular, creamy-yellow, opaque, smooth, entire	Gram +ve cocci in cluster	<i>Staphylococcus sp</i>
IR2	Cream, circular, smooth, raised and lobate	Gram +ve cocci in cluster	<i>Staphylococcus sp</i>
IR3	Cream, circular, opaque, flat, entire, rough	Gram +ve, long rod with central spore.	<i>Bacillus sp</i>
IR4	Cream, circular, flat, rough and lobate	Gram +ve, long rod	<i>Bacillus sp</i>

Table 2: Biochemical and morphological characteristics of isolated organisms from the condiments (okpei, ogiri, ugba, iru.)

Probable organism present.	Man-itol	Ara	Suc	Lact	Mal	Fruc	Gluc	Starch
<i>Bacillus-subtilis</i>	AG	AG	A	A	A	AG	AG	+
<i>Bacillus-cereus</i>	-	-	G -	A	A	A	+	
<i>Lactobacillus-brevis</i>	-	A	AG	-	-	A	AG	-
<i>Lactobacillus-fermenti</i>	AG	-	A	A	-	AG	AG	-
<i>Bacillus-subtilis</i>	AG	AG	A	A	A	AG	AG	+
<i>Micrococcus-variens</i>	-	AG	AG	AG	-	A	AG	+
<i>Bacillus-subtilis</i>	AG	AG	A	A	A	AG	AG	+
<i>Micrococcus-roseus</i>	-	-	A	-	-	A	A	+
<i>Staphylococcus-aureus</i>	AG	G	AG	A	AG	AG	AG	+
<i>Staphylococcus-saprophyticus</i>	-	-	A	A	-	A	AG	-
<i>Bacillus-subtilis</i>	AG	AG	A	A	A	AG	AG	+
<i>Bacillus-cereus</i>	-	-	G	-	A	A A	+	

Probable organism present	Indole	Gelatine	Coagu-lase	Catalase	Motility	Spore location	Gram stain	Isolate
<i>Bacillus-subtilis</i>	-	+	NA	+	+	Central spore	+	OK1
<i>Bacillus-cereus</i>	+	-	NA	-	+		+	OK2
<i>Lactobacillus-brevis</i>	-	-	N	+	-		+	OK3
<i>Lactobacillus-fermenti</i>	-	+	NA	-	-	-	+	OG1
<i>Bacillus-subtilis</i>	-	+	NA	+	+	Central spore	+	OG2
<i>Micrococcus-variens</i>	-	-	-	+	+	NA	+	OG3
<i>Bacillus-subtilis</i>	-	+	NA	+	+	Central spore	+	UG1
<i>Micrococcus-roseus</i>	-	-	-	+	+	NA	-	UG2
<i>Staphylococcus-aureus</i>	-	+	+	+	-	NA	+	IR1
<i>Staphylococcus-saprophyticus</i>	-	-	+	-	+	NA	+	IR2
<i>Bacillus-subtilis</i>	-	+	NA	+	+	Central spore	+	IR3
<i>Bacillus-cereus</i>	-	-	NA	-	+		+	IR4

Foot Note: - = negative, AG = Acid and Gas, Glu=Glucose, Ara = Arabinose, OK = Okpei, IR = Iru, + = Positive, A = Acid, Mal = Maltose, Suc = Sucrose, UG = Ugba, NA = not applicable, G = Gas, Lact = Lactose, OG = Ogiri.

has been associated with fermenting locust bean for iru production (Antai and Ibrahim, 1986) and for fermenting soy bean for natto production (Hesseltine, 1965). Although *Staphylococcus sp.* and *Micrococcus sp.* were isolated from the fermented bean, they were present in low numbers compared to *Bacillus sp.* They did not appear to be important in the fermentation process. The organisms isolated from fermented castor oil seed (ogiri) were species of *Bacillus*, *Micrococcus* and *Lactobacillus*. As in the case of iru, the predominant organisms observed were *Bacillus sp.* *Lactobacillus* was present probably because temperatures above 22°C favour its growth. *Lactobacillus* produces acid, which further inhibits the growth of non desirable organisms. The organisms isolated from ugba were species of *Bacillus* and *Micrococcus*. *Bacillus* species were the predominant microorganisms present. This

agrees with the observation of Isu and Njoku (1997) that *Bacillus* species constitute over 95% of the total microbial population density in ugba fermentation. This may be because *Bacillus* cells exhibit very high protease activity compared with the other bacteria isolates. Organisms isolated from fermented mesquite seeds were *Bacillus sp.* and *Lactobacillus sp.* Like the other products discussed earlier, the *Bacillus sp.* were predominant with the other organisms adding little or no value to the product.

Biochemical and morphological characteristics of isolates: *Bacillus sp.* Are known to utilize the three major constituents of raw oil bean seeds, that is, protein, carbohydrate and oil very well because they are important sources of lipolytic enzymes as well as proteases and amylases (Pederson, 1979). Tables 1

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Table 3: Mean scores of the sensory evaluation of the fermented products*

(a) "IRU" Mean Score			
Sample Code	Colour	Aroma	Texture
IR1	4.0 ^a	4.2 ^a	4.0 ^a
IR2	3.9 ^a	3.8 ^{ab}	4.0 ^a
IR3	2.6 ^b	2.9 ^b	3.0 ^b
IR4	3.0 ^b	3.6 ^{b^c}	3.5 ^{bc}

IR1 = sample fermented with *Staphylococcus aureus*

IR2 = sample fermented with *Staphylococcus saprophyticus*

IR3 = sample fermented with *Bacillus subtilis*

IR4 = sample fermented with *Bacillus cereus*

(b) "OGIRI" Mean Score

Sample Code	Colour	Aroma	Texture
OG1	4.3 ^a	4.1 ^a	3.8 ^a
OG2	2.7 ^b	3.3 ^b	2.4 ^b
OG3	3.7 ^a	4.0 ^a	3.2 ^c

OG1 = sample fermented with *Lactobacillus fermenti*

OG2 = sample fermented with *Bacillus subtilis*

OG3 = sample fermented with *Micrococcus varians*

(c) "OKPEI" Mean Score

Sample code	Colour	Aroma	Texture
OK1	3.09 ^a	2.8 ^a	4.0 ^a
OK2	3.8 ^b	3.4 ^b	4.3 ^a
OK3	2.4 ^{ab}	3.8 ^b	4.3 ^a

OK1 = sample fermented with *Bacillus subtilis*

OK2 = sample fermented with *Bacillus cereus*

OK3 = Sample fermented with *Lactobacillus brevis*

(d) "UGBA" Mean Score

Sample code	Colour	Aroma	Texture
UG1	2.3 ^a	2.2 ^a	3.4 ^a
UG2	4.2 ^b	4.0 ^b	4.5 ^b

Ug1 = sample fermented with *Bacillus subtilis*

Ug2 = sample fermented with *Micrococcus roseus*

*Means with the same letter(s) in a column are not significantly different.

and 2 show the biochemical and morphological characteristics of the isolated organisms. *Bacillus subtilis* and *Bacillus cereus* are similar morphologically but biochemically, *Bacillus subtilis* is arabinose positive while *Bacillus cereus* is negative to the test (Turchetti, 1982). *B. subtilis* is indole negative while *B. cereus* is positive to the test. *B. subtilis* is positive to manitol while *B. cereus* is not. *B. subtilis* hydrolyses gelatine while *B. cereus* does not hydrolyze it. *Micrococcus varians* and *Micrococcus roseus* are similar morphologically and to a great extent biochemically. Pigmentation is the only character by which *M. roseus* can be distinguished from certain other micrococci (Cowan and Steel, 1966). *M. roseus* consists of strains with a common feature in producing a pink pigment, but may differ in biological character. The *Micrococcus sp.* are typically non motile and catalase positive and aerobic in nature. The *Staphylococcus sp.* are similar morphologically, but biochemically, *S. aureus* hydrolyses gelatine while *S. saprophyticus* is negative to the test. Also *S. aureus*

hydrolyses starch while *S. saprophyticus* does not. *Lactobacillus sp.* also differ biochemically. *L. brevis* is catalase positive while *L. fermenti* is negative to the test. *L. fermenti* hydrolyses gelatine while *L. brevis* does not hydrolyze it. *L. fermenti* is positive to manitol test while *L. brevis* is negative to the test.

Sensory evaluation of the products fermented by different identified organisms:

Significant differences exist among the various organisms in their ability to ferment locust bean seeds, castor oil seeds, mesquite seeds and African oil bean seeds (Table 3). Among the iru samples, the color of IR3 (fermented by *Bacillus subtilis*) was the most preferred by the judges followed by sample IR4. The color may have been developed by the microbial activity during the fermentation process. Sample IR3 gave the preferred iru aroma at ($P = 0.05$). Samples IR1 and IR2 were not significantly different and were rated poorer than others. There was a slight difference between samples IR3 and IR4. Sample IR3 was significantly different from samples IR1 and IR2 and was rated better than others in terms of product texture. The results show that *Staphylococcus* species were not involved in the fermentation. Among the ogiri samples, OG2 was significantly different from samples OG1 and OG3. The color of OG2 (fermented by *Bacillus subtilis*) was most preferred, followed by that of sample OG3. There was no significant difference between samples OG1 and OG3. The aroma of Sample OG2 was most preferred and this could be attributed to the action of the fermenting organisms. The okpei sample OK1 (fermented by *Bacillus subtilis*) was preferred by the judges to the other samples in terms of colour, aroma and texture. There was no significant difference between samples OK2 and OK3. The results show that *Lactobacillus brevis* and *Bacillus cereus* did not contribute much to the fermentation. Among ugba samples, UG1 was preferred by the judges. It gave the desired colour, aroma and texture of ugba at $P = 0.05$.

Conclusion: Results of the present study show that the following organisms were isolated from the respective fermented oil seed samples. Iru-*Staphylococcus aureus*, *Staphylococcus saprophyticus*, *Bacillus subtilis* and *Bacillus cereus*. Ogiri-*Lactobacillus fermenti*, *Bacillus subtilis*, and *Micrococcus varians*. Okpei-*Bacillus subtilis*, *Bacillus cereus* and *Lactobacillus brevis*. Ugba-*Bacillus subtilis* and *Micrococcus roseus*. *Bacillus subtilis* was found to be common to all of them. It fermented the seeds very well and gave the desired colour, texture and aroma to the respective seeds fermented. Overall, all the judges preferred the samples that were fermented with *Bacillus subtilis* to those fermented with the other isolated organisms. Therefore, it could be concluded that *Bacillus subtilis* is the predominant organism

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responsible for the fermentation of protein-rich oil seeds to give the desired fermented products (ogiri, iru, ugba and okpei)

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