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Research Paper

Assessment of Biochemical and Antimicrobial Activity of Bacteria Associated with Fermented Locust Beans in Manyare Community of Kogi State, Nigeria

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A fermented locust bean (*Parkia biglobosa*) is one of the most popular food condiments in region of Western and Southern African. It is one of the major sources of plant protein in African diet. Raw seed were prepared in the traditional African way by boiling them for 6 h to soften the seed coat; and for another 1 h to soften the cotyledon. The boiled seeds were immediately transferred into a basket line with banana leaves and wrapped tightly to prevent heat loss. They were left at ambient temperature to ferment for 72 h. Total bacterial community of the seed was obtained by taking one gram and mashed with mortar and pestle, dissolve in 9 ml of distilled as diluents, then ten-fold serial dilution (10^{-2}, $10^{-3,}$ 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8}, 10^{-9} and 10^{-10}) were made from this solution. Nutrient Agar was prepared and 0.1.ml of the inoculums was taken, inoculated and incubated at 30°C for 24 h. The bacterial isolates were identified by standard microbiological measures. The organisms isolated include; *Bacillus subtilis, Bacillus cereus, Staphylococcus aureus and Staphylococcus saprophyticus.* The *in vitro* antibiotic susceptibility testing of bacterial isolates was performed using the standardized disc agar diffusion. The physical characteristics observed in the organoleptic assessment include; color, taste, aroma and texture. The major constituents of fermented locust beans include; proteins, fats and carbohydrates. With the ever increasing cost of protein sources in Africa and other developing countries, the ferment is recommended for use as food condiment especially in soups to alleviating protein deficiencies and its associated diseases.

Keywords: Locust bean, organoleptic, biochemical, antimicrobial, assessment.

INTRODUCTION

Fermented locust beans (*Parkia biglobosa*) are one of the most popular food condiments in region of West and Central Africa (Campbell-Platt, 1980). This nutritious and delicious food spice is popularly called “ogiri” in Igbo “iru” in Yoruba, “bindo” in Bassa or “dadawa” in Hausa in Nigeria. It is used to enhance food flavour and nutritional values. It is heavily consumed in Nigeria, Ghana, Sierra Leone and Togo (Odunfa, 1985). Locust beans are a dicotyledonous angiosperm belonging to the family “Fabaceae-Mimosoideae”. It is categorized under spermatophytes, vascular plants (Thiombiano *et al.*, 2012). It is deciduous perennial tree that grows to between 7 and 20 m high. In some cases, up to 30 m (Ntui *et al.*, 2012). The tree is a fire-resistant helophyte characterized by a thick dark gray-brown bark (Abioye *et al.*, 2013). The pods of the tree are pink in color in the beginning and turn dark brown when fully mature. They are also 30-40 centimeters long on average, with some

reaching lengths of about 45 centimeters. Each pod can contain up to 30 seeds (Janick, 2008).

The tree is not normally cultivated but can be seen in population of two or more in the savannah region of West Africa (Hopkins, 1993). Locust bean tree is an indigenous tree with economical and social importance to the African people. The locust bean is prepared locally by using the seed inside the pod of the locust beans tree. Locust beans are produced by a natural un-inoculated solid-substrate fermentation of the boiled and dehulled cotyledon, the major fermenting organism are the *Bacillus* and *Staphylococcus* species. The fermentation of the seeds makes them edible by increasing their digestibility (Odunfa and Oyewole, 1986). At some stages in the preparation of the seed fermentation is required to bring out the desire nutritional value and other organoleptic properties such as taste, flavour and texture.

Locust beans have been reported to be rich in fat (39 to 40%) and protein (31 to 40%) and contribute significantly to the energy intake, protein and vitamins especially in riboflavin, in many countries of Central and West Africa (Ademola *et al.,* 2011). Other biochemical changes that occur during locust bean fermentation include the hydrolysis of indigestible oligosaccharide present in *Parkia biglobosa*, notably stachyose and raffinose to simple sugars by alpha and beta galactosidase, the synthesis of B-vitamins (thiamin and riboflavin) vitamins C and the reduction of anti-nutritional factors (oxalates and phytates) (Orwa *et al.,* 2009).

Due to the importance of this condiment among the Manyere community of Kogi state, this study aim to assess the biochemical and antimicrobial activity of bacteria associated with fermented locust beans from this community.

MATERIALS AND METHODS

MATERIALS

The matured and dried fruits pods were randomly collected from different branches of ten locust bean trees at farmlands of Manyare community of Kogi State. This was label as Sample A. Fermented locust beans was bought from two different local traders of the product in Manyare community which was also labeled as B1 and B2 respectively. The samples were transported to University of Abuja, Microbiology Laboratory in airtight polyethylene bag.

Processing of the locust bean seeds (*Parkia biglobosa*).

Traditional method of processing locust beans was used and their unit operations were closely monitored especially at the critical control points. The method used by local processor in Manyare community was employed. The raw seeds were cleaned to remove impurities like shafts and stones, washed in pot. The seeds were then cooked for about 6 h until over 50% of the seeds coat cracked. Excess water drained off using a sieve and the seeds were dehulled by pounding gently in a cyclical manner in a wooden mortar to separate the seed coat from the cotyledons.

The cotyledons were severally and thoroughly washed in water and then sieved to remove the non-cotyledon materials. The cotyledons were parboiled for 1 h. The colour of the cotyledons changed from whitish yellow to light brown at this stage.

Rubber sieve was used to drain the boiling water before fermenting the cotyledons. The sterilized cotyledons were poured into a basket line with banana leaves and covered tightly immediately to prevent the heat from escaping. The basket was wrapped with several thick clothes and then fermented in a dark, warm and moisture free environment for three days (72 h). At the end of fermentation, the colour of the cotyledons changed to ash white (Figure 1).

Sterilization of equipments

All equipments used for the experiment were sterilized. The test tubes and other glassware were sterilized using the autoclave at 121°C for 15 min. Other materials used such as glass spreader were heat-sterilized using Bunsen flame. The work benches were chemically sterilized by swabbing their surfaces with 70% ethanol to ensure aseptic condition for the experimental procedures.

Isolation of microorganisms

One gram each of sample A, B1, and B2 were taken aseptically and were thoroughly mashed with 9 ml of distilled water as diluents in bijous bottles and the contents were thoroughly shaken. Subsequent serial dilutions (10^{-2} , 10^{-3}, 10^{-4} 10^{-5}, 10^{-6}, 10^{-7}, 10^{-8} 10^{-9}, 10^{-10}) were made from this solution by adding serially 1 ml of solution from preceding concentration to 9 ml of the diluents, using sterile syringes.

The nutrients Agar was prepared by adding 250 ml of distilled water to 7.0 g of agar in a conical flask. The content was thoroughly shaken to fully dissolve all components.

The mixture was autoclave at 121°C for 15 min. Into 9 Petri-dishes 3 to 5 ml of the sterile warm (45 °C) nutrient agar was poured. The plates were labeled appropriately. 0.1 ml of the inoculums was taken from 10^{-2}, 10^{-4}, 10^{-6} each of sample A, B_1 and B_2 and introduced into the plates containing nutrients agar, inverted and incubated at 30°C for 24 h in an incubator according to Victor (2011) method.

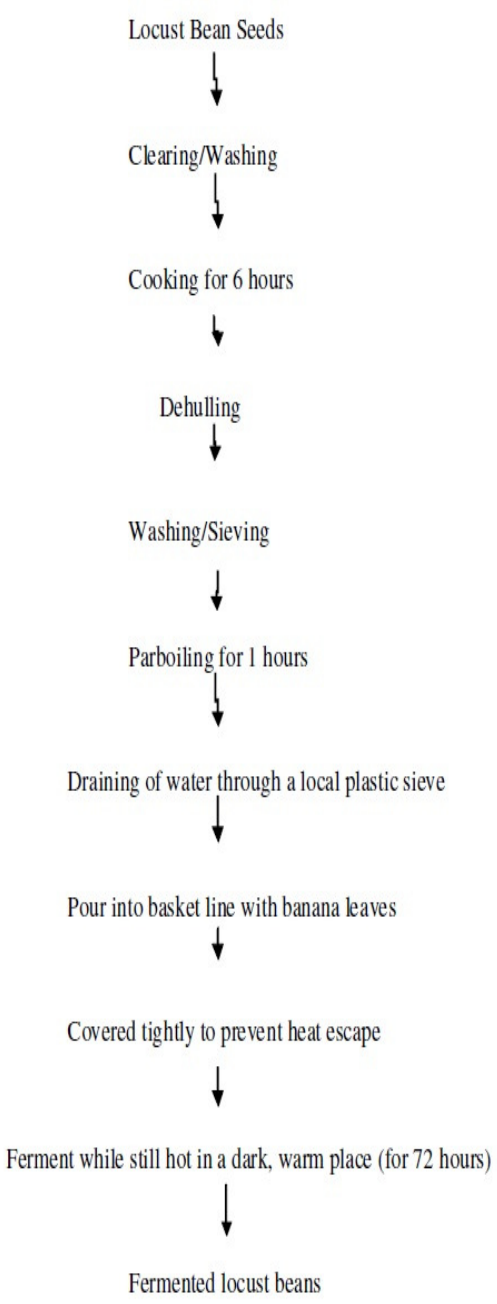

Figure1. Traditional processing of making fermented locust beans according to method used by (Odunfa and Oyewole, 1986).

Characterization and identification of isolates

Colonies obtained after incubation were sub-cultured on nutrient agar which was incubated for 24 h at 30°C. The cultural characteristics of isolates on the agar plates were observed. Stock cultures from this source were prepared in nutrient agar slants and then kept in the refrigerator at 4°C for further use. The bacterial isolates were identified by standard microbiological measures. The motility of the isolates was examined using hanging drop technique. The characterization of bacteria isolates were based on Gram staining, morphological and cultural characteristics coupled with relevant biochemical tests. 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).

Antibiotic sensitivity test

The *in vitro* antibiotic susceptibility testing of bacterial isolates was performed using the standardized disc agar diffusion methods of the (CLSI, 2013; Kaiser, 2012). The test was carried out 18 h by using peptone H_2O culture of each isolate incubated in test tube at 37°C. After incubation a sterile swab stick was used to pick a suspension of the test organism in the test tube and was spread evenly across the plates containing Muller Hinton agar. A sterile forceps was used to place the antimicrobial disc between the medium and the culture incubated at 37°C for 18-24 h. The test was read after checking the bacterial zones of inhibition around the disc.

Statistical analysis

Mean zones of inhibition values obtained for the antibiotic sensitivity testing of bacterial isolates of *Parkia biglobosa* were subjected to one-way analysis of variance (ANOVA) at $p>0.05$ level of significance for comparison of the antibiotics activities on the test organisms.

RESULTS

The results on the investigation of biochemical assessment and antimicrobial activity of bacteria associated with fermented locust beans are shown in (Tables 1-4).

Table 1 shows the colony characteristics of the isolates. The physical characteristics observed in the organoleptic assessment include; colour, taste, aroma and texture.

The results in (Table 2) shows the different organisms isolated from locally produced locust bean in Manyare Community of Kogi State and the one prepared aseptically at Microbiology Laboratory, University of Abuja, while (Table 3) shows the biochemical and morphological characteristics of isolated organism from *Parkia biglobosa*.

The antibiotic sensitivity pattern of bacterial isolates using ten tips multiple disc are recorded in (Table 4).

Table 1. Colony Characterization of the Isolates.

Isolates	Colony morphology	Characterization	Probable Identification
A	Creamy, opaque, entire, rough, Wide, regular, very large, rod, with central spore, raised, dry, slight	Gram positive, long	*Bacillus subtilis*
B1	Irregular, creamy-yellow, opaque, Smooth, moderate, entire	Gram positive cocci in cluster	*Staphyloccocus saprophyticus*
B2	Scanty, irregular, creamy, Transparent, raised, dry, entire, large	Gram positive rod	*Bacillus cereus*

Key: A= Laboratory fermented locust beans B1 and B2 = Locally fermented locust beans.

Table 2. Microorganisms Isolated from locally and laboratory prepared *Parkia biglobosa* (Locust beans).

Isolates	Locally prepared locust beans	Laboratory prepared locust beans
B. subtilis	+	+
B. cereus	+	-
S. saprophyticus	+	-
S. saureus	+	+

Key: += present, - = Absent

DISCUSSION

Since the major constituents of fermented locust beans are proteins, fats and carbohydrates, the organisms responsible for fermenting them must be capable of utilizing these three constituents. All the organisms isolated from the fermented locust beans are known to posses such characteristics. The organisms isolated from fermented locust beans were *Bacillus* and *Staphyloccocus* species. *Bacillus* speices were the predominant microorganism present. These are known to have proteolytic ability and are also able to break down oil (Forgarty *et al*., 1974). The report of this research is in agreement with the findings of Antai and Ibrahim, (1986) where *Bacillus subtilis* was associated with fermenting locust bean for Iru production. Although *Staphylococcus* species were isolated from the fermented bean, they were present in low number compared to *Bacillus* species. This may be because *Bacillus* cells exhibit very high protease activity compared with other bacteria isolates.

Table 1 and 2 show the biochemical and morphological characterization of the isolated organisms. *Bacillus subtilis* and *Bacillus cereus* are similar morphologically but biochemically they are different. *Bacillus subtilis*is is arabinose positive while *Bacillus cereus* is negative to the arabinose test (Turchetti, 1982). *Bacillus subtilis* is indole negative while *B. cereus* is positive to the test. *B. subtilis*is also positive to manitol while *B. cereus*is not. *B. subtilis* hydrolyses gelatin while *B. cereus* does not hydrolyze it. Also, *Staphylococcus* species are similar morphologically, but differ in some biochemical tests. *S. aureus* hydrolyses gelatin and starch while *S. Saprophyticus* is negative to the tests.

Owen *et al.* (1997) on studying the aroma and flavour characteristics of Bacillus fermented *P. biglobosa* noted the presence of active aldehydes, ketones, and acids. Odunfa and Adewuyi, (1985) and Campbell-Platt, (1980) also noted a high level of peptides and amino-acids.

Locust beans are a protein-rich fermented African soup condiment which is considered the most important food condiment in the entire West African Savannah region. Its production is however basically by traditional household level fermentation technology laden with food-borne hazards at virtually all the points during processing as observed in this study. The food condiment is however populated with some groups of bacteria (Table 1). Critical control points and possible food safety risk in the processing of *Parkia biglobosa* into Iru were identified with the one produced in the laboratory during this study. It is expected that the longer the period of fermentation (4-5 days), the better the product, due to more activities of the fermenting bacterial flora, but period of fermentation is another major difference in the method of traditional production of Iru, because nowadays most of the fermentation times are usually one to three days instead of four to five days. The result obtained in this research corroborates with the study of Liman *et al.* (2010) who demonstrated the impacts of some environmental conditions on quality of processed locust beans.

Bacillus species which are the predominant organisms obtained in this study are capable of increasing the protein and fat content of samples significantly because

Table 3. Biochemical and Morphological characteristics of isolated organisms from locust beans (parkiabiglobosa).

Identification	*Staphylococcus aureus*	*Staphylococcus saprophyticus*	*Bacillus subtilis*	*Bacillus cereus*
Maltose	A	A	A	A
Catalase	+	_	+	_
Glucose	+	+	+	+
Arabinose	G	_	AG	_
Lactose	+	+	_	_
Sucrose	_	+	_	_
Manitol	AG	AG	A	A
Fructose	AG	AG	AG	AG
Citrate	_	_	_	_
Motility	+	+	+	+
Gram stain	+	+	+	+
Gelatine	+	+	+	_
Indole	-	-	-	+
Spore location	NA	NA	Central spore	_
Starch	_	+	+	_
Coagulation	+	+	NA	NA
Isolates	A	B_1	A	B_2

Table4. Diameter of zones of Inhibition of Antibiotic Sensitivity Pattern of Bacteria Isolates (in mm).

Bacteria species	AM	CPX	SXT	APX	CN	PEF	R	S	E	Z
Staphylococcus Saprophyticus	-	11R	12R	-	-	-	-	-	-	-
Staphylococcus aureus	-	20S	13R	-	15S	12R	-	-	-	-
Bacillus subtilis	-	10R	-	-	11R	-	-	10R	-	-
Bacillus cereus	-	18S	-	13R	-	-	-	-	-	-

Key; AM = Amoxacillum, APX = Ampiclox, CPX = Ciprofloxocin, E = Erythromycin, CN = Gentamycin, PEF = Pefloxacin, R = Rocephin, SXT = Septrin, S = Streptomycin, Z = Zinnacef. R= Resistance, S= Susceptible and - =No reaction.

these organisms have proteolytic and lipolytic ability (Table 3). This is consistent with the study of Ogbadu and Okagbue (1988), which shows that various *Bacillus* species were responsible for the fermentation of *P. biglobosa* seeds. The presence and use of these groups of organisms, as a starter culture for the product may be desirable because of their probiotics potential. However, the determination of some commonly used antibiotics against isolates from this sources which shows multiple antibiotics resistance (Table 4) is worrisome and hence the need to protect this condiment preparation under better hygienic processes.

Conclusion

The result of the present study shows that the following organisms were isolated from fermented locust beans- *Staphylococcus aureus, Staphylococcus saprophyticus, Bacillus subtilis* and *Bacillus cereus.*The physical characteristics observed in the organoleptic assessment include; color, taste, aroma and texture. The major constituents of fermented locust beans include; proteins, fats and carbohydrates. With the ever increasing cost of protein sources in Africa and other developing countries, the ferment is recommended for use as food condiment especially in soups to alleviating protein deficiencies and its associated diseases. It is recommended that the users of fermented locust bean should cook it well before eaten and ensure to obtain it from a hygienic environment.

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