

Isolation and Identification of Fungi in Fermented Locust Beans (*Parkia Biglobosa*) Preserved by Different Storage Methods

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Abstract

An experimental study was carried out to isolate and identify fungi fermented locust Beans (*Parkia biglobosa*) preserved by different storage methods. Samples of locust beans (*Parkia biglobosa*) obtained from an open market in Lagos metropolis, were subjected to four different storage methods namely refrigeration, Oven drying at 50 ° C, sun drying and storage under room temperature for two months. Samples were cultured for presence of fungi using a locally prepared potatoes dextrose agar (PDA) under good hygienic Conditions using already made manufacturer's specification with modifications. The Incubation period lasted for 2 to 4 weeks. The fungi growth ranges between 2.5×10^6 (\log_{10} 2.39) to 6.5×10^7 (\log_{10} 7.8). Pure Cultures were examined macroscopically and microscopically, Culture characteristics like texture, Colour, size, morphology of the spore produced and shape of the upper thallus and production of pigment on underside noted. Fungi from positive Cultures were Identified based on Colonial characteristics, Cultural and morphological features such as pigment production, Conidial morphology, Colonies appearance and microscopic examination in Lacto phenol cotton blue preparation. Isolation of the fungi was done in an inoculation site Swabbed with ethanol before inoculation. The agar was inoculated by transferring some. Locust beans (*Parkia biglobosa*) to Surface of the medium using Sterile spatula. The following fungi *Zygosaccharomyces rouxii*, *Debaryomyces hansenii*, *Aspergillus flavus*, *Penicillium* spp., *Aspergillus niger*, *Rhizopus* spp, *Fusarium* spp and *Alternaria alternata* were isolated and identified in fermented locust beans (*Parkia biglobosa*) preserved using different Storage methods. The highest fungi isolate and identified was recorded in fermented locust beans (*Parkia biglobosa*) preserved by room temperature storage method, While Sample preserved by oven drying Storage method had the least fungi Isolate and Identified. Result shows that no method of storage totally preserves locust beans (*Parkia biglobosa*) against fungi growth. It was therefore recommended among others that Oven drying Storage method is far better than other Storage methods

Keywords: *Isolations; Identifications, Fungi, Locust beans, Storage, Method.*

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Background to the Study

A condiment is a spice sauce that is added to food to impart a particular flavor; to enhance its flavor or in some cultures to complement meals. Many diverse condiments exist in various countries, regions and culture. According to Collins (2014), the term condiment was coined from the Latin word 'condimentum' which means spice seasoning and sauce. Also coined from Latin word 'condere' meaning preserve, and pickle season. Spices and condiments are important components of food because they significantly change the taste, appearance and effects when used in small quantities. Many condiments promote digestion; impact little effect on acid base balance; and have more health benefits (Aluko *et al*; 2021). Leguminous seeds account for up to 80% of dietary protein (Nucara; 2022) The cooked forms leguminous seeds are eaten as meals or fermented form as condiments to enhance flavours of foods. (Chukwu *et al*; 2020)

Locust beans (*Parkia biglobosa*), known as the African locust bean is a dicotyledonous, perennial deciduous tree of the Fabaceae family grows to between 7 and 20 metres high, in some cases up to 30 metres (Ogaraku; 2010). The tree of the locust bean requires “between 0-300 metres of altitude, a mean annual rainfall of between 400-700 millimetres and a mean annual temperature of about 24-28°C.” It prefers well-drained, thick clay soils but can also be found on shallow, thin sandy soils. It is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. Where the tree is grown, the crushing and fermenting of these seeds constitutes an important economic activity. Various parts of the locust bean tree are used for medicinal purposes. The locust bean tree is also important in medicinal practices in treatment of ailments such as bronchitis, pneumonia, malaria, diarrhoea and as poison for sore eyes (Farombi; 2003). Although microorganisms of all groups including bacteria, protozoa, algae, viruses, fungi together with insects and rodents play significant role in food deterioration, the most active and more versatile organisms that affect locust bean seeds and its products causing spoilage when stored are species of bacteria and fungi (Omafuvbe *et al.*, 2000). They can occur on growing crops as well as harvested commodities leading to damage ranging from rancidity, odour and flavour changes and germ layer destruction (Cutler; 1991). In a study to identify the bacterial and fungal flora of deteriorated and maggot infested samples of fermented locust bean seeds, the isolated fungal species were identified as *Aspergillusniger*, *Aspergillusflavus*, *Penicillium*, *Rhizopus* and *Candida* species. *Parkia biglobosa* seeds are subject to degradation induced by diverse organisms including fungi which are among the most active microorganisms in these processes (Popoola and Akueshi, 1985). Microorganisms associated with fermented locust bean seeds have been widely studied (Odunfa, 1981; Ikenebomehet *al.*, 1986; Odunfa and Oyewole, 1986; Ogbadu and Okagbue, 1988). Bacilli and Staphylococci were observed to dominate the fermentation together with a number of fungal species causing deterioration of this especially in storage in Northern Nigeria.

The tree, locust beans (*Parkia biglobosa*), is used locally and internationally for drug and cosmetics production. The populations of locust beans (*Parkia biglobosa*) has continues to

decrease in the wild because it's undomesticated nature (Ojewumi, 2016). Fermented locust beans (*Parkia biglobosa*) commonly refer to as 'Locust beans' is called different names in different countries of tropical Africa In Sierra Leone, it is called 'Kinda'; 'Iru or Dawadawa' in Nigeria and Ghana, (Modupe, Abiodun, & Adesola, 2016); 'Afintin and Sonru' in Benin Republic, (Azokpota, 2005); and 'Natto' in Japan. In Nigeria, locust beans (*Parkia biglobosa*) is known as 'Iru' by Yoruba; 'Dadawa' by Hausa, and 'Opei' by Igbo. Locust beans is fermented, processed and used as a condiment in cooking. It is popular among Yoruba people of South Western part of Nigeria. It is used for cooking traditional soups. The nutritious and delicious food spice is popularly called “ogiri” in Igbo, “iru” in Yoruba and “dawadawa” in Hausa in Nigeria. It is heavily consumed in Nigeria, Ghana, Sierre Leone and Togo (Odunfa, 1985). It serves as source of protein for most of the people whose protein intake is low due to high cost of animal protein sources. The Yoruba have classified locust beans (*Parkia biglobosa*) into two types; loose type (Iru wooro) which is commonly used in making stew. The blended type (Iru pete) is used in making vegetable soup. They are processed into fresh or dried form. The dry form is flattened into disc or cakes for sale while the dry form “is weaker in flavor and pungency. The dry form stores better than fresh form. Studies (Emmanuel *et al.*, 2022; Olojede and Akintunde, 2019; Jide *et al.*, 2018) have revealed that locust beans are high in lipid (29%), protein (35%) and carbohydrates (16%). It is a good source of calcium and fat. According to Adewale (2017), locust beans promote good sight and drives hypertension and other disease condition like stroke and diabetes. Locust beans contain tannins, astringent substance found in many plants (Pizzi 2021; Salem *et al.*, 2021). Food rich in tannins are often recommended for treatments of diarrhea (Pizzi 2021).

The tree of locust beans is employed in wound healing and serves as one of the ingredients used in treating leprosy (Adewale, 2017). Also, locust bean seed for controlling blood cholesterol level and promotion of digestion (Karyem et al 2023). The water and alcohol extracts of locust bean are useful for reduction of blood sugar and management of bacterial infection (Younes et al 2023). Till date, traditional and fermentation process of locust beans production are done using indigenous microflora derived from immediate environment (Olabiwonninu, Olaoluwa & Odunfa, 2017). Locust bean is regarded as ecological resource rich in nutrients and capable of attracting bacterial and fungal colony growth. Bacterial and fungal colonization of locust bean products normally alter food taste, and nutritional value, structure and quality thereby causing food spoilage. Food spoilage is accompanied by production of toxic secondary metabolites which may result in serious medical problem.

In other cases, colonization with number fungi is beneficial with respect to nutritional value and prolonged storage of food products due to fermentation. Food spoilage is responsible for enormous food losses worldwide and a major threat to human health in Nigeria. The processing, transportation, storage, display and sales expose locust beans and products to fungal contaminations. These fungi may be normal microflora. Some fungi are agents of spoilage and cause diseases. This study therefore seeks to isolate and identify of fungi associated with fermented locust beans (*Parkia biglobosa*) using different storage methods.

Materials and Methods

The fermented locust beans (*Parkia biglobosa*) was purchased from an open market in Lagos metropolis. The samples were collected into a sterilized plastic bowl and covered to avoid exposure to moisture and contaminant. The samples were transported to laboratory for chemical analysis. Four samples weighing 100g locust beans (*Parkia biglobosa*) each were placed in sterilized plastic bowls with covers and labelled 1 to 4 according to storage methods as follows:

Simple 1: Storage in refrigerator at -10°C

Simple 2: Storage in on laboratory table at room temperature.

Simple 3: Storage by sun drying to reduce moisture content to 5%.

Simple 4: Storage by oven drying at 50°C to reduce moisture content to 5%.

All labeled samples except simple 1 in refrigerator were left under laboratory room temperature for a period of 60 days. Each set comprises of five replicates. To prepare culture Potato Dextrose Agar (PDA) medium, freshly purchased potato were peeled, sliced into pieces and washed under running water in laboratory. Then, 100g potato was weighed into 500ml beakers into a cleaned pot, 200ml distilled water added and boiled to softness. The soft potato was put in a mortar and pestle to form paste. The paste was put in 500ml beaker, mixed with distilled water and filtered into a beaker until one litre (1000ml) filtrates collected. 10g dextrose and 15g of agar were added to the filtrate and mixed thoroughly. The mouth conical flasks containing mixture was plugged with a non-absorbent cotton wool wrapped with aluminium foil.

The resulting medium was autoclaved at 95 °C for 15 minutes, and 1.1kg/cm pressure. The mixture was allowed to cool, and sterilized pH of the prepared medium adjusted with 10% lactic acid at the rate of 25ml per litre. This reduced pH of the medium to about 4.8 which is low enough to inhibit growth of most bacteria and fungi. The medium was allowed to cool in a water bath to form Potato Dextrose Agar (PDA) medium. It was then poured into sterilized petri-dishes and allowed to solidify before use. Isolation of fungi was done in an inoculation site swabbed with 75% ethanol before inoculation. The agar was inoculated by transferring some of locust beans (*Parkia biglobosa*) to surface of the medium using a sterile spatula. The plates were labeled and incubated for three weeks at 27-30°C. To obtain pure cultures, sub-culture was done on a Plain Potato Dextrose Agar (PPDA).

All necessary precautions were taken to prevent contamination during isolation. Culture plates without growth were discarded as negative after four weeks of inoculation. Identification of fungi in positive cultures was based on colonial characteristics, cultural and morphological features such as pigment production, conidial morphology, colonies appearance and microscopic examination in lacto phenol cotton blue preparation. A small quantity of mycelia of pathogens from pure culture was taken with a hot sterilized inoculating needle and placed on a sterilized microscopic glass slide having passed slightly over a flame. A drop of lacto phenol in cotton blue stain was added and sterile

cover slip placed on it. Excess stain was wiped off with a cotton wool. The slide was carefully examined under low (x10) and high (x40) power objectives to observe fungal colonies using a microscope.

A well-prepared slide display fungus clearly showing vegetative and reproductive part. The photomicrograph of slide was taken by attaching a camera (Motic Mac Camera [2000] 2.0 megapixel digital coloured camera) connected to computer for microscopic photograph of fungi. The pure culture of fungi obtained was identified by the principles of morphological appearance, spores formation and production of fruiting bodies as appeared under microscope (Alexopoulos, 1996; Dugan, 2006). The identified fungi were also compared with already known fungal species.

Results

Plates 1 and 2 show the growth of fungi (*Aspergillus niger*) on locust beans after subjected to oven drying and sun drying storage methods respectively.



Plate 1: *Aspergillus niger* on oven dried sample



Plate 2: *Aspergillus niger* on sun dried sample



Plate 3: *Rhizopus spp.*, at a room temperature

Table 2 below shows total fungi counts after five days of incubation period in different samples stored by sun drying, oven drying, refrigeration and room temperature

Table 2: Total fungi counts of incubation period using different storage methods

S/N	Method of storage	Fungi counts (Cfu/mg)
1	Sun drying	3.5×10^5 ($\log_{10}5.54$)
2	Oven drying	2.5×10^2 ($\log_{10}2.39$)
3	Refrigeration	4.5×10^3 ($\log_{10}3.65$)
4	Room temperature	6.5×10^7 ($\log_{10}7.8$)

The highest fungi growth was recorded in fermented locust beans stored under room temperature without adding any preservative. This sample has larger amount of moisture content and richer nutrient bases to support fungi growth. The moisture content was reduced by sun heat but that does not stop fungi growth.

Sun drying method when spread under sun could possibly expose sample to contamination of fungi spores present in atmosphere by wind current. Also, the atmospheric temperature could be high enough to dry completely moisture content within a day or two. Length of exposure to atmospheric spores is a major factor that increased fungi growth. It is not because water content in the sample is the only factor responsible for the growth of fungi but growth recorded was due to contamination during sun drying.

Oven drying storage method recorded the least fungi growth due to the fact that drying took place in an enclosed place without exposure to environment where it can be contaminated either by spores from air, human and environmental induced factors. However, no matter how hygienic one maintained during food processing and handling fungi or other microorganisms could be found in food. Microorganisms are present everywhere and there is no sterile environment even in extreme situation. For this is the reason, food standard and safety regulation agencies are established. Refrigerated samples recorded slightly high in numbers 4.5×10^3 ($\log_{10} 3.5$). This is not expected because cold temperature is expected to suppress growth of fungi. The level of growth could be due to problem of epileptic electricity supply during which temperature in the refrigerator could increase.

The power supply could become erratic during storage and allow fluctuation of hot and cold in refrigerator, the fungi can thrive hence the high fungi growth. Also, fungi present in market samples could remain dormant until environment condition becomes favourable for growth. The different identified fungi in samples of locust beans (*Parkia biglobosa*) stored using sun drying, oven drying, refrigeration and room temperature storage methods is presented in Table 3.

Table 3: Fungi isolates in locust beans stored by different methods

S/N	Method of storage	Fungi isolates
1	Sun drying	<i>Zygosaccharo mycesrouxii</i> , <i>Zygosaccharo mycesbailii</i> , <i>Debaryo myceshanseni</i> , <i>Aspergillus flavus</i> and <i>Penicillium notatum</i>
2	Oven drying	<i>Aspergillus niger</i> , <i>Aspergillus flavus</i> , <i>Rhizopus Species</i> , <i>Mucor spp</i> and <i>Fusarium spp</i> .
3	Refrigeration	<i>Mucor species</i> , <i>Aspergillus niger</i> , <i>Alternaria spp</i> , <i>Aspergillus flavus</i> and <i>Aspergillus fumigates</i>
4	Room temperature	<i>Penicillium niger</i> <i>Saccharomyces cerevicea</i> and <i>Aspergillus flavus</i>
		<i>Alternaria spp</i> , <i>Rhizopus</i> , <i>Fusarium spp</i> --- <i>niger</i> , <i>Aspergillus falvus</i>

There is clear indication that *Aspergillus spp* are predominant fungi found in fermented locust bean (*Parkia biglobosa*) followed by yeast of *Zygosaccharo mycesrouxii*, *Zygosaccharo mycesbailli*, *Debaro myceshanseni* and *Saccharomyces cerellicea*.

Discussion

All samples collected from the markets showed fungal infection. A total of 9 fungal isolates were isolated during the study. The fungi isolates included. The genera of mycotoxigenic fungi mainly *Aspergillus*, *Penicillum*, *Fusarium* and *Alternaria* were isolated in this research work. Also, higher numbers of isolates were recorded on fermented locust beans (*Parkia biglobosa*) stored under room temperature without adding any preservative. In a related study Kumasi *et al* (2009), (Molnár *et al.* 2015) and (Yahaya *et al.*, 2018) observed that many of the post-harvest diseases of grains and legumes are the result of infections by pathogens in the field which continue to develop after harvest. Fungi isolated included Aflatoxins are potent carcinogenic and in association with Hepatitis B virus are being responsible for many thousands of human deaths per annum (Richard; 2007). Aflatoxin contamination in foods has become serious public health issue in 2004 in Kenya, 125 people were killed and nearly 200 others were treated after eating aflatoxin contamination food (Adeyeye; 2016). The 2004 outbreak occurred from widespread aflatoxin contamination of locally grown maize stored under damp conditions. A similar cases of food poisoning of toxicity due to mycotoxins occurred in several countries in Africa. Therefore, similar situation can occur in locust beans (*Parkia biglobosa*) since they are produced and stored in damp condition. Oma Uaboi-Egbenni, P. O fuvbe *et al.*, (2000) and Bigneil (2010) reported that species of bacteria and fungi affect locust bean seeds and its products causing spoilage when stored. Grains readily spoil due to microbial activities and are generally short lived unless steps are taken to remove, kill or prevent growth of associated microorganism (Omafuvbe *et al.* 2000; Richard *et al.* 2017). The pathogens can occur on growing crops as well as harvested commodities leading to damage ranging from rancidity, odour and flavour changes and germ layer destruction. In a related study on maize grain (Adamu ;2012) to develop after harvest (*Parkia biglobosa*) Omafuvbe *et al.*, 2016) and (Bigneil 2018) reported that species of

bacteria and fungi affect locust bean seeds and its products causing spoilage when stored. Grains readily spoil due to microbial activities and are generally short lived unless steps are taken to remove, kill or prevent growth of associated microorga (*Parkia biglobosa*) nism (Omafuvbe *et al.* 2000; Richard1 *et al.* 2017) [20, 21]. The pathogens can occur on growing crops as well as harvested commodities leading to damage ranging from rancidity, odour and flavour changes and germ layer destruction. In a related study on maize grain (Adamu, 2015)

Aspergillus flavus one of the isolates has been associated with various diseases such as aflatoxicosis in domestic animals and humans throughout the world (Ashiq 2015). Also, *Fusarium* produce fumonisins cause equine *leukoencephalomalacia*: swelling of the lungs and thorax. Fermented foods have good safety record in developing world where foods are manufactured by people without training in microbiology or chemistry (Renuka *et al*;2024) However, fermented foods cannot solve the problems of contaminated drinking water and improper personal hygiene by food handlers. The different studies (Okoro,et al;2023) have shown that fungi contamination of locust beans appeared to be normal resident of condiment, while some indicate inherent danger to consumer of the product. Uaboi-Egbenni, P. O (2009) reported the isolation of *Aspergillus niger*, *Aspergillus rapens*, *Aspergillus flavus*, *Aspergillus fumigatus* and *Rhizopus stolonifer* at higher level, although none of the fungi was associated with fermentation process of the product but as a result of contamination due to poor handling during processing, distribution and when display for sales. Also, poor packaging and storage add to contamination level. Ponchang (2015) reported isolation of seventeen fungal species with *Aspergillus species* being the most dominant. The author noted that fungi growth impaired negatively on quantity of fermented locust beans such that protein content reduced by 9.07%; fibre by 3.52%; and carbohydrate content by 23.37%.

Apart from *Aspergillus* being dominant the *species*, other fungi isolate according to the researcher were *Mucor species*, *Penicillium species*, *Rhizopus species*, *Cladosporium species*. The fungi isolates have serious health implication as some of them produce mycotoxins carcinogenic agents which are elaborated into the food substance. Fungi are able to grow on fermented locust beans (*Parkia biglobosa*) irrespective of the storage methods. The level of growth is determined by moisture level, exposure to environment, and handling. Storage by refrigeration does not ensure total preservation from fungal growth. Fungi not only cause deterioration in quality of locust beans (*Parkia biglobosa*) but also produce secondary metabolites called mycotoxins which have serious health consequences. These mycotoxins are reported to be heat stable and are dangerous to human system when ingested in small concentrations. As supported by (E.Okolo *et al*;2023)

Aspergillus niger have been reported as opportunistic pathogens causing asthma and ostomycosis in compromised individuals (Yakasia *et al*, 2021). Also, *Aspergillus* and *Penicillium* have been reported to impact negative odour on product thus causing huge economic loss. (Niego *et al*; 2023)

Conclusion

The medicinal and the nutritional value benefits of Locust beans by humans cannot be over emphasized. Hence, the need for proper preservation of locust beans to avoid fungi infection is very important. This study reviews that **oven** drying method is the best method of preserving locust beans (*Parkia biglobosa*) when compared with the following preservation methods, Oven drying method, Sun drying method, Refrigerator method, and Room temperature method before storage

Recommendations

Based on the results obtained from the present study, the following recommendations are made

- a. In preserving locust beans, the oven drying method is the most effective when compared with
- b. Oven dried locust beans should be store in a dry cool place in a sealed container to avoid accumulation of moisture and thereby preventing the growth of microbes such as fungi.
- c. Spoilt locust beans should not be consumed by humans rather should be discarded to avoid health complications.
- d. More research should be conducted to ascertain the safety shelf life of locust beans for human consumption.

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