

SAFETY ASSESSMENT OF FERMENTED AFRICAN LOCUST BEAN SEED (*PARKIA BIGLOBOSA*) IN OGBOMOSO MARKET, OYO STATE, NIGERIA

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ABSTRACT

Locust beans have been utilized as condiment (seasoning agent, flavor enhancer) from time immemorial. It is produced locally and so can be purchased in the local market all over Nigeria. The local processing operation which does not have standard control procedure has been known to attract various hazards which can affect its qualities. As a result, microbiological study of each unit operation is necessary to assess the hazard points and possible attention and remedy.

Samples of the locust beans were collected at each of the processing steps of the unit operation. The bacterial populations of the samples were estimated using pour plate technique, identification of the bacterial isolate by standard microbiological methods (disc agar diffusion method). Characterization of bacteria isolates were based on gram staining, morphological, cultural characteristics and biochemical test. The antibiotic susceptibility profile of the isolate was also determined.

The bacterial load obtained ranges from 0.50 TVC X 10⁸ to 32.0 TVC X 10⁸. The number of distinct colonies obtained per plate ranges from 2.0 to 5.0 colonies. The Statistical analysis showed that *Staphylococcus aureus* and *Bacillus cereus* have the highest frequency of occurrence (19.40 %), *Pseudomonas auraginosa* and *staphylococcus epidermis* have the lowest occurrence (4.48 %). Bacterial isolated include *Staphylococcus aureus*, *Staphylococcus epidermis*, *Bacillus subtilis*, *E. coli*. Among the organisms isolated *Staphylococcus aureus*, *Bacillus subtilis*, *Clostridium spp*, *Bacillus cereus* and *Pseudomonas aeuraginosa* showed multiple Antibiotic Resistance. The percentage MAR ranges between 22-44 %.

This study showed that there is a need for improvement in the processing methods starting from the collection of seeds on the farm to the final product.

KEYWORDS: Fermentation, Locust Bean, Characterization, Identification, Processing Method

INTRODUCTION

Locust beans condiment (iru- Yoruba, Daddawa- Hausa, Ogiri- Igbo) is utilized as delicacy, used as food seasoning, flavor enhancer and aroma. It is produced locally and can be purchased in local markets all over Nigeria (Rabi, *et al.*, 2013). African locust bean (*Parkia biglobosa*) is a nutritious as it is rich in protein and some other beneficial food

components. It serves as a cheap source of protein for most of the people whose protein intake is low due to high costs of animal protein sources or for those who do not eat meat (Ajayi, 2014). Previous studies (Oke and Umoh, 1987; Elemo, *et al.*, 2011) showed that locust bean is highly digestible (74% - 97%). This is coupled with its high commercial values as food and a medicinal agent (Gernah, *et al.*, 2011). Lipolysis and proteolysis are very important for the quality of African locust bean-based condiments fermented by *Bacillus spp.* (Obadu, *et al.*, 1988). Proteolysis has been reported as the main metabolic activity during the fermentation of African locust bean (Allagheny, *et al.*, 1996).

Fermented food condiments give a pleasant aroma to soups, sauces and other prepared dishes worldwide, especially in most African countries and India, where protein calorie malnutrition is a major problem (Sarkar, *et al.*, 1993; Enujiugba, *et al.*, 2008; Lima, *et al.*, 2010). Dawadawa is a typical condiment for flavoring of meals such as stew and soup made from soybean by the Hausa tribe in Nigeria that is similar to fermented locust beans (*Parkia biglobosa*). It is also valuable food condiment, which adds aroma and flavor to dishes such as jolof rice, vegetable soup, fish pepper soup and chicken pepper soup (Yakubu, 2009).

Exploring the spices and condiments, identify their properties and find out how to match them with other ingredients is a great task especially the local spices and condiments. It should be noted that herbs, spices and condiment are meant to compliment cooking ingredients and to enhance the natural tastes and flavors of food but not to mask the natural tastes of the foods, hence it required real skill to be able to strike the balance. Some of the commonly use local herbs, spices and condiments are: Locust beans (iru, dadawa, or dawadawa), Scent leaves (sweet basil), Achii, Ogbono, African black pepper, Crayfish, Garlic, ginger, Alligator pepper, onions and Bitter leaf (Koleoso, 2013).

In West/Central Africa savannah region, fermented locust bean constituted 1.4% of the daily calorie intake and 5% of the total protein intake (Odunfa, *et al.*, 1985; 1986). Fermented food stuff and condiment are very popular in Nigeria, prominent among the soup condiment is “iru”, a fermented vegetable protein from locust bean seeds (*Parkia biglobosa*), Oil seeds such as African locust bean, melon seed, castor oil seed, mesquite bean and soybean are also fermented to give condiments. In many cases, fermentation is responsible for the development of taste and aroma, improve digestibility, improvement of nutritional composition, stabilization of the original raw materials and detoxification of anti-nutrient factors in the products (Olayinka and Fawole, 2006).

In traditional process, water used for “iru” processing is usually the surface water sources, which can introduce contaminations into the peeled cotyledons. Similarly, the critical hazard points of public health which not usually properly control includes: feet used for de-hulling the seed cotyledons, fermentation trough, addition of salt in open places and wrapping leaves. Different type of leaves are use for rapping which includes banana, teak (*Tectona grandis*) leaves, etc. these leaves are usually picked from the ground around the shedding trees for wrapping the product and can serve as contaminants. Hence, the microbiological and food safety caution is necessary. In this study, various bacterial species associated with some locally fermented products in locust beans were isolated and identified. Moreover investigation on microbiological hazards associated with fermented locust beans was also intensified to safeguard the production of this food condiment meant for human consumption.

Materials

Samples were collected from two different productions sources: local production centers at Ogbomoso in Oyo state and Eyenkorin at Ilorin in kwara state. The samples were taken from every unit operation stages of locust beans

production. Media used include nutrients Agar from Lab M. Limited UK. Lactobacillus (MRS Agar) from Titan Biotechnology Ltd. The equipment used are: incubator (Gallenkamp, USA), Weighing balance (Mettler Teledo Pb 3002 Switzerland), Hot air oven (Gallen Kamp), Hotplate and Autoclave.

Methods

The samples were taken aseptically at every stage of the processing unit of production as showed in the flow chart. Samples were transferred to the laboratory for analysis. The laboratory control sample was prepared to evaluate the critical control point.



The Nutrient Agar was weighed according to manufacturers' directions. Lactobacillus (MRS Agar) was prepared for use according to the manufacturers' instruction.

The Isolation and Identification of Bacteria after serial dilution were carried out by weighing 1 gm of sample into 10 ml of diluents water to form a stock culture. 1 ml of appropriate diluents was cultured in nutrient agar plates at 37⁰ C for 24 hrs. The bacteria population of the locust bean samples was estimated from pouring plate technique. Stock cultures from these samples were prepared in nutrient agar slants and kept in the refrigerator for further use. Identification of the bacterial isolate was carried out by standard microbiological methods. The characterization of bacteria isolates were based on gram staining, morphological and cultural characteristics couple with relevant biochemical tests.

The antibiotic sensitivity test of the bacterial isolates was carried out using the standard disc agar diffusion methods of CM-12-8PR100 and CM-12-8NR100. The test was carried out using Muller Hinton agar incubated in plates at 37⁰ C for 18 h. the bacterial susceptibility was determine by zone of inhibition.

RESULTS AND DISCUSSIONS

The result of microbial analysis of iru samples are presented in the tables below. The result showed that there are different microorganisms which are normal micro-flora that are involve in the fermentation processes. The number of

common floral found in fermented “iru” include *Lactobacillus species*, *Bacillus spp*, *Staphylococcus aureus*. Table 1 showed that the microbial load of dry clean seeds was highest at center, with TVC of 3×10^3 and the lowest is TVC 0.6×10^3 obtained at center 1 (Table 1). Boiled peel seed has the highest value of TVC (28×10^3), lowest value (3×10^3). Cooked seed TVC highest value is 18×10^3 , and the lowest value is 2×10^3 , Iru woro has TVC of 24 as the highest and the lowest value s 5×10^3 . Salted iru- woro highest value is 2×10^3 and the lowest value 1×10^3 , Iru-pete highest value 21×10^3 , and the lowest value of 10×10^3 . According to Ajayi, (2014), the result of this project is similar to previous works where the common bacterial species that persistently populate the samples includes *Bacillus spp* and *Staphylococcus spp*.

In the first center sampled, *Staphylococcus aureus* was isolated from sample A1, B1, C1, D1, E1, F1. *Bacillus cereus* was isolated from A1, B1, C1, E1 and F1. *Bacillus subtili* was isolated from E1 and F1. *Clostridium spp* was isolated from D1 and F1. *Lactobacillus spp* was isolated from D1, E1 and F1 respectively. *Pseudomonas aeruginosa* was isolated from sample B1, and C1. In center two, *E. coli* was isolated from sample D2 and E2. *Staphylococcus epidermis* was found in sample A2 and C2. *Staphylococcus aureus* was found in sample A2, C2, D2 and F2. *Clostridium spp* was found in A2, B2, C2, and D2 also, *Acetobacter spp* was found in sample A2, C2, and E2 while *Lactobacillus species* was isolated from sample A2 and C2. The isolates obtained in sample center three are *Staphylococcus aureus* from sample A3, D3 and E3, *Bacillus cereus* was isolated from sample A3, B3, C3. *Bacillus subtilis* was isolated from D3 and E3. *Clostridium spp* was isolated from sample A3 B3, C3. *Acetobacter spp* was isolated from sample A3, C3 and E3. *Lactobacillus spp* was isolated from sample A3 and C3 while *Pseudomonas aeruginosa* was isolated from sample B3, as presented in Table 2. Table 3 showed cultural and biochemical characterization of the bacterial isolates encountered in samples. Table 4 showed the susceptibility of the isolates to antibiotic, *Staph aureus*, *Bacillus subtilis*, *clostridium spp* *Pseudomonas aeruginosa* showed Multiple Antibiotic Resistance (MAR). According to Osundiya *et al.*, (2013), the antibiotic increasingly compromises the outcome of many infections that were, until recently, treatable and remain the most common disease in Africa (Okeke *et al.*, 2005). Many authors support the fact that *P. aeruginosa*, *Staph. aureus* is implicated as commonly found resistant to antibiotics as identified in this work (Lambert 2002; Okeke *et al.*, 2005; Magiorakos *et al.*, 2012; Osundiya *et al.*, 2013). Table 5, presented frequency of occurrence in percentage, *Staphylococcus aureus* and *Bacillus cereus* has the highest occurrence followed by *Clostridium spp*, and the least occurrence is *Pseudomonas aeruginosa*.

CONCLUSIONS

This research showed that there are various types of microorganisms involved in fermentation of locust bean seeds. Diverse groups of bacteria isolates obtained include: *Bacillus*, *Lactobacillus*, *Enterobacteraceae* and *E. coli*.

In addition, it may be further concluded that every stage of production is sensitive regarding the load of microorganisms, therefore, there must be proper control to prevent contaminations. Heating process must be thoroughly carried out, rinsing must be done with clean treated water. The personnel involved must have good personal hygiene. The local producer must be trained on good manufacturing practices.

Table 1: Microbial Population at Different Stages of Local Production at Collection Center

Nos.	Sample	Code	TVC1 X10 ⁸	TVC2 X10 ⁸	TVC3 X10 ⁸
1	Dry clean seed	A	0.6	2	3
2	Boiled peel seed	B	28	7	3
3	Cooked seed	C	18	6	2
4	Iru woro	D	24	24	5
5	Iru woro+ salt	E	1.2	2	1
6	Iru pete	F	21	20	10

Table 2: Incidence of Isolation of Sample from Different Location

Bacterial Isolates	A	B	C	D	E	F
Staphylococcus aureus	+++	+(1)	++(1,2)	+++	++(1,3)	++(1,2)
Staphylococcus epidermis	+(2)		+(1)		+(2)	
Bacillus cereus	+++	+++	+++	+(D)	++(1,3)	+(1)
Bacillus subtili				++(2,3)	+++	++(1,2)
Clostridium spp	++(1,3)	++(2,3)	++(2,3)	++(1,2)		+(1)
E. coli			++(1,2)	+(1)	+(1)	+(1)
Acetobacter spp	++(2,3)		++(2,3)		++(2,3)	+(1)
Lactobacillus spp	++(2,3)		++(2,3)	+(1)	+(1)	+(1)
Pseudomonasaeruginosa		++(1,3)	+(1)			

Key: A- Dry clean seed, B-Boiled peel seed, C- Cooked seed, D-Iru woro, E-Iru woro+salt, F-Iru-pet

Table 3: Cultural and Biochemical Characterization of the Bacterial Isolates Encountered in Sampled African Locust Bean

Isolates code	Cultural characteristics	Gram Reaction	Motility test	Spore staining	Capsule stain.	Catalase test	Methyl red test	Starch hydrolysis	Cl rate at litmit in	Oxygen react in	Lactose	glucose	sucrose	Mintose	fructose	Identification
DLIa	Raised,opaque, smooth, creamy white clusters	Gm + Coccus in cluster	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus aureus
DLIb	Translucent,raised lobate	Gm+ rod in chain	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	Bacillus cereus
DLIc	Flat, opaque, white, dull, lobate	Gram + rod in clusters	-	+	+	+	+	-	+	FAN	AG	A	A	A	A	Bacillus subtilis
DLId	Raised, opaque, cream, entire, rough	Gram+ve rod in chain	+	+	-	-	-	+	-	AN	A	A	A	A	A	Clostridium spp
DLIe	Raised, translucent, cream, lobate, rough	Gram -ve rod	-	-	-	-	-	-	-	AN	A	A	-	-	-	E-coli
LSEa	Flat, opaque, white, lobate dull	Gram +ve rod in cluster	-	+	+	+	+	-	+	FAN	AG	A	A	A	A	Bacillus
LSEb	Raised, translucent, cream, lobate, rough	Gram -ve rod	-	-	-	-	-	-	-	AN	A	A	-	-	-	Acetobacter spp
LSEc	Raised, opaque, creamy white, entire, smooth	Gram +ve cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus aureus
LSEd	Raised, translucent, yellowish, entire, smooth	Gram -ve rod in chain	-	-	-	+	-	+	+	AE	-	A	-	-	-	Pseudomonas aeruginosa
LSEa	Raised, opaque, creamy white, entire, smooth	Gram +ve, cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus aureus
LSEb	Raised, opaque, creamy white, entire, smooth	Gram + ve, cocci in clusters.	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus aureus
LBSc	Raised, opaque, creamy white, entire, smooth	Gram + ve, cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus epidermis
LSBd	Raised, opaque, creamy white, entire, smooth	Gram +ve, cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus aureus
LBSe	Raised, translucent, cream, lobate, dull	Gram +ve, rod in chain	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	Bacillus cereus
LBSf	Flat, opaque, white, lobate, dull	Gram +ve, rod in clusters.	-	+	+	+	+	-	+	FAN	AG	A	A	A	A	Bacillus subtilis
LBSg	Raised, opaque, cream, entire, rough	Gram -ve rod in chain	-	+	-	-	-	+	-	AN	A	AG	A	AG	A	Clostridium species
LBFa	Raised, translucent, cream, lobate, dull	Gram +ve, rod in chain	-	+	+	+	+	+	-	AE	-	AG	A	AG	AG	Bacillus cereus
LBFb	Flat, opaque, white, lobate, dull	Gram +ve, rod, clusters	-	+	+	+	+	-	-	FAN	AG	A	A	A	A	Bacillus subtilis
LABa	Raised, opaque, creamy white, entire, smooth	Gram +ve, cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	Staphylococcus aureus
LABb	Raised, translucent, cream, lobate, rough	Gram -ve rod	-	-	-	-	-	-	-	AN	A	A	-	-	-	Acetobacter species
LABc	Raised, opaque, creamy white, lobate, smooth	Gram +ve, rod clusters	-	-	-	-	-	+	-	FAN	A	A	A	A	AG	Lactobacillus species

Lab Code	Appearance	Gram	+	-	+	-	+	-	+	-	+	-	+	-	+	Species
LABd	Raised, opaque, creamy, entire, rough.	Gram -ve, rod in chain.	-	+	-	-	-	+	-	AN	A	AG	A	AG	A	<i>Clostridium species</i>
LABe	Raised, translucent, cream, lobate, dull.	Gram +ve, rod in chain.	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	<i>Bacillus cereus</i>
DABa	Raised, opaque, creamy white, entire, smooth.	Gram +ve, cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	<i>Staphylococcus aureus</i>
DABb	Flat, opaque, white, lobate, dull.	Gram +ve, rod in clusters	-	+	+	+	+	-	+	FAN	AG	A	A	A	A	<i>Bacillus subtilis</i>
LIDa	Raised, opaque, creamy white, entire, smooth.	Gram+ve, cocci in closters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	<i>Staphylococcus aureus</i>
LABa	Raised, translucent, cream, lobate, rough	Gram-ve, rod	-	-	-	-	-	-	-	AN	A	A	-	-	-	<i>Acetobacter species</i>
LABb	Raised, opaque, creamy white, lobate, smooth.	Gram+ve, rod in clusters	-	-	-	-	-	+	-	FAN	A	A	A	A	AG	<i>Lactobacillus species</i>
LABc	Raised, opaque, cream, entire, rough	Gram -ve, rod in chain.	-	+	-	-	-	+	-	AN	A	AG	A	AG	A	<i>Clostridium species</i>
LABd	Raised, translucent, cream, lobate, dull.	Gram+ve, rod in chain	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	<i>Bacillus cereus</i>
LABe	Raised, translucent, yellowis, entire, smooth.	Gram -ve, rod in chain	-	-	-	+	-	+	+	AE	-	A	-	-	-	<i>Pseudomonas aeruginosa</i>
LABf	Raised, opaque, cream, entire, rough	Gram -ve rod chain	-	+	-	-	-	+	-	AN	A	AG	A	AG	AG	<i>Clostridium species</i>
LABg	Raised, translucent, cream, lobate, dull.	Gram +ve, rod chain	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	<i>Bacillus cereus</i>
LBDa	Raised, opaque, cream, entire, smooth.	Gram +ve, cocci in clusters	-	-	-	+	-	+	+	FAN	AG	A	A	A	+	<i>Staphylococcus aureus</i>
LBDb	Raised, translucent, creamy, lobate, dull.	Gram +ve, rod in chain	-	+	+	+	+	+	+	AE	-	AG	A	AG	AG	<i>Bacillus cereus</i>
LBDc	Flat, opaque, white, lobate, lobate, dull.	Gram +ve, rod clusters.	-	+	+	+	+	-	+	FAN	AG	A	A	A	A	<i>Bacillus subtilis</i>
LBDd	Raised, opaque, cream, entire, rough.	Gram-ve, rod in chain.	-	+	-	-	-	+	-	AN	A	AG	A	AG	A	<i>Clostridium species</i>
LBDe	Raised, translucent, yellow, entire smooth.	Gram -ve rod in chain	-	-	-	+	-	+	+	AE	-	A	-	-	-	<i>Pseudomonas aeruginosa</i>

Key: - Negative, AE – Aerobic, AN- Anaerobic, A- Acid production, + - Positive, FAN – Facultative anaerobe, AG – Acid and Gas production, YC- Yellow cream

Table 4: Antibiotic Sensitivity /Susceptibility Profile of Isolates (Mm)

Lab Code	Bacterial spp	OFL	CXC	ERY	CTR	GEN	CRX	CAZ	AUG	CPR
DLIa	<i>Staph. aureus</i>	18S	10R	ND	ND	11R	NQ	ND	ND	ND
LABb	<i>Acetobata spp</i>	15S	ND	ND	ND	ND	ND	ND	ND	ND
LASf	<i>Bacillus subtilis</i>	12R	ND	10R	11R	16S	ND	ND	11R	ND
LABc	<i>Clostridium spp</i>	16S	10R	15S	ND	ND	10	10	10	ND
LADc	<i>Bacillus cereus</i>	13I	ND	10R	ND	11R	11R	12R	ND	ND
LABe	<i>Pseudomonas aeruginosa</i>	119S	ND	10R	10R	20S	ND	15S	20S	ND
LABc	<i>Lactobacillus spp</i>	ND	ND	11R	14S	ND	ND	ND	ND	ND

Key: ND- not determined, S- Sensitive, I- Intermediate, R- Resistant

Table 5: Microorganisms and Frequency of Occurrence Table

S. NO	Organisms	Frequency	Percentage (%)
1	<i>Staph. Aureous</i>	13	19.400
2	<i>Acetobacter species</i>	7	10.451
3	<i>Bacillus subtilis</i>	7	10.451
4	<i>Clostridium spp</i>	9	13.437
5	<i>Bacillus cereus</i>	13	19.400
6	<i>Pseudomonas aeruginosa</i>	3	4.479
7	<i>Lactobacillus spp</i>	7	10.451
8	<i>E. coli</i>	5	7.467
9	<i>Staphylococcus epidemis</i>	3	4.479

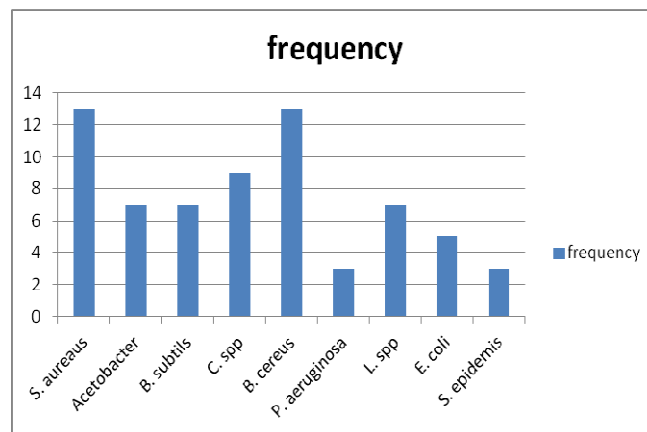


Figure 1: Bar Chart Representing Frequency of Occurrence of Bacteria Isolate

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