ASSESSMENT OF EFFECT OF DIFFERENT PACKAGING MATERIALS ON MICROBIAL QUALITY OF LOCUST BEANS DURING STORAGE

ABSTRACT

This study assesses the effect of different packaging materials on the microbial quality of Africa Locust beans during storage. Locust beans (Dawadawa) also known as iru, among the Yorubas in South-west Nigeria, is a popular condiment used as taste and flavour enhancer in soup and dishes in Africa. Locust bean is traditionally produced from locust beans seed (*Parkia biglobosa*) and preserved using different packaging materials before use in order to prolong its shelf life. The Microbiological examination of the produced locust beans was carried out on samples wrapped with different packaging materials such as plastic container, nylon and dry banana leaves. The suspected organisms isolated include *Salmonella spp, Staphylococcus aureus, Streptococcus Lactis, Pseudomonas aeruginosa, Lactobacillus plantarium, Bacillus cereus, Bacillus subtilis, Leuconostoc sp, Escherichia coli* and *Micrococcus sp.* It was observed from this study that locust bean packaged with banana leaves had the highest microbial load, few of which are of public health concern while plastic container had least microbial load. It is therefore recommended that locust beans should be produced and packaged with sealed plastic containers to avoid contamination by pathogenic organisms.

INTRODUCTION

African locust beans (*Parkia biglobosa*) also known as iru, among the Yorubas in South-west Nigeria, is a popular condiment used as taste and flavour enhancer in soup and dishes in Africa.

Dawadawa is traditionally produced from locust beans (*Parkia biglobosa*) seeds (Farinde *et al*., 2014). Odunfa (2010) stated that fermented locust bean seed is commonly consumed in Ghana, Nigeria, Sierra Leone and Togo. In Nigeria it is called iru in Yoruba, dawadawa in Hausa and ogiri `igala in Igbo. It is also referred to as "kinda" in Sierra Leone and kpalugu in Ghana. Preservation and preservatives are designed to inhibit/control the activities of spoilage causing organisms in food, a process also referred to as sanitization. African locust bean is a nutritious source of food since it is rich in protein and some beneficial health components. It serves as a cheap source of protein for people whose source of protein is low due to high cost of animal protein sources (Jay, 2010).

Food packaging is an integral part of food processing, which entails the use of some materials in the wrapping of foods (Ayo-Lawa *et al*., 2016). The success of most preservation methods depends on appropriate packaging. However, faulty packaging will undo all that a good processor has attempted to accomplish by the most meticulous manufacturing process (Ihemeje *et al*.,2022).

Spoilage causing organisms due to their growth and metabolic activities produce by-products, which change the texture, taste, flavor and the aroma of the food (Jay, 2010). The use of packaging and preservation of locust beans is to make the product shelf life stay fresher and longer. Dehydration, salting and packaging of home-produced food items such as locust beans (iru) in simple polyethylene bags is desirable to extend their shelf life. (Farinde *et al*., 2014).

In Nigeria, African locust bean (*Parkia biglobosa*) tree grows widely throughout the savanna (Akoma *et al*., 2001). A mature pod contains yellow, dry and powdery pulp in which dark brown seeds are embedded. The pulp is licked for its sweet taste but only to a small extent.

The pulp is usually washed away when the seeds are processed into condiment called dawadawa or iru. Dawadawa is a source of protein intake among the low-income groups and rural populations of West Africa. While the seed has been extensively studied (Addy *et al*., 1995), little has been done on the utilization of the pulp. In West Africa, the pulp is prepared as flour and used in soups and stews or eaten with cereals as porridge (Omauvbe *et al*., 2004).

Plants are known to contain high amounts of essential nutrients, vitamins, minerals, fatty acids and fibre (Gafar and Itodo, 2011). Plants also contain other chemical compounds such as saponins, tannins, oxalates, phytates, trypsin inhibitors and cyanogenic glycosides, which are known as secondary metabolites and are biologically active (Soetan and Oyewole, 2009). The seeds on fermentation are used in cooking stew and soup. It has been reported that the husks and pods are good for livestock (Obizoba, 1998). The roots, barks, leaves, stems, flowers, fruits and seeds of *P. biglobosa* are all used medicinally to treat a range of ailments, including diarrhea ulcers, pneumonia, burns, coughs, etc (Sacande and Clethero, 2007).

Different packaging materials are used for wrapping locust beans in order to extend its shelf life and the microbial load of these packaging materials are mostly not assessed due to ignorance and little information on its effect on the product. It is therefore essential to assess the effect of different packaging materials used for the storage and preservation of the locust beans. This will ascertain the best packaging materials with the lowest microbial load suitable for its preservation. This will give information on how to improve the shelf life of locust beans and encourage its acceptability

MATERIALS AND METHODS

Sample Collection and Preparation

Parkia biglobosa (Africa locust bean) seed used for this research were purchased from Oja-Oba market in Ado-Ekiti, Ekiti-State, Nigeria.

Sample Preparation

The production and fermentation process were carried out in the department of Food Technology laboratory, School of science and computer studies. Federal polytechnic Ado-Ekiti, Ekiti-State Nigeria

Production of fermented *Parkia biglobosa* (Africa locust bean) "iru" using traditional method.

Parkia biglobosa (Africa locust bean) seed was prepared according to the method of (Audu *et al.*, 2004). *Parkia biglobosa* (Africa locust bean) seed were sorted and washed to remove the yellowish pulp from the seeds. The locust bean seed was cooked with pressure pot. Put on electric cooker for 7 hours, with water being added frequently to it, until the seed becomes softer, then the seed was allowed to cool down and it was pounded by using mortar and pestle, the cotyledon was separated from its coat. Inside a bowl of water by using hand and sieve then the seed was washed and cleaned thoroughly. It was then re-cooked for another 1hour 30minutes with pressure pot, until the seed becomes softer. Then the water was drained off by using sieve. The locust bean was spread into a big sieve wrapped with multiple cloth to maintain a hot atmosphere to be fermented for 72hours. After fermentation the locust bean "iru" was packaged in three different materials (Banana leaf, Plastic rubber and nylon) and preserved for 7 days.

Media Preparation

The media (Nutrient agar, MacConkey agar, Salmonella shigella agar and Eosin Methylene blue agar) used for this research were prepared according to the manufacturer's instruction. The prepared media were poured into conical flasks and the flasks were covered with cotton wool and Aluminum foil. The media were sterilized and autoclaved at 121^0c for 15minutes and used for microbial analysis (Temple, *et al., 2006*).

Microbiological Analysis of Fermented Sample

One gram of the sample (Fermented *Parkia biglobosa* (Africa locust bean) were taken respectively and marched using mortar and pestle for proper homogenization. One gram of sample was diluted serially in seven folds dilution blanks and properly mixed with sterile glass rod. 0.1ml of diluted sample was introduced into sterile plates and agar. The media was poured at 45^0C. The plates were rotated gently to disperse the inoculum in medium and allowed solidify. Then the plates were incubated at 37°C and the resulting colonies of microbes were sub-cultured to get the colonies in their pure form and to lower the density of cells. The culture was maintained as slants on both nutrient Agars. The slants were kept and stored for further use.

Examination of Culture Plates of Isolated

Macroscopic: the colonial appearance of the organism was noted such as colour, shape and size after 24hours of incubation.

Characterization and identification of sample

Gram Staining reaction was carried out by emulsifying one isolate 24hour old colony in a drop of water placed at the center of a clean grease tree slide passed through a Bunsen burner. The heat fixed smear was flooded with crystal violet for 60seconds, after which the stain was poured off the slide and rinsed with running tap water. The smear was flooded with iodine.

Identification of Bacteria Isolates

The bacterial isolates were identified by using morphological and biochemical tests such as coagulase test, catalase test, oxidase test, indole test, urea production, nitrate reductions, motility test, spore test, hydrogen sulfide production test, oxidase production, citrate utilization test, nitrate reduction test, Voges Proskauer and Methyl red test. (Fawole and Oso, 2004) and Olutiola *et al*. (2000)

RESULTS

The results of the biochemical characterization of the microorganisms isolated from locust bean wrapped with different packaging materials such as plastic container, nylon and dry banana leaves are shown in the tables below.

Table 1: Biochemical characteristics of isolates from African Locust beans package with plastic container

Isolates	Grams Rxn	Shape	Catalase	Indole	Vp	Oxidase	Methyl red	Citrate	Probable organisms
1	-	rod	+	+	-	-	+	+	*Salmonella spp*
2	+	Cocci in cluster	+	+	-	+	+	+	*Staphylococcus Aureus*
3	-	rod	+	-	-	+	+	+	*Pseudomonas aeruginosa*
4	+	rod	+	-	+	-	-	+	*Bacillus*

									Cereus
5	+	rod	+	-	+	+	-	+	*Bacillus subtilis*

Key:

+ means positive

- Means negative

Table 2: Biochemical characteristics of isolates from African Locust beans packaged with nylon

Isolates	**Grams Rxn**	**Shape**	**Catalase**	**Indole**	**Vp**	**Oxidase**	**Methyl red**	**Citrate**	**Probable organisms**
1	-	rod	+	+	-	-	+	+	*Salmonella spp*
2	+	Cocci in chains	-	-	-	+	+	-	*Streptococcus Lactis*
3	-	rod	+	-	-	+	+	+	*Pseudomonas aeruginosa*
4	+	Bacilli	-	-	-	-	-	-	*Lactobacillus plantarium*

5	+	rod	+	-	+	-	-	+	*Bacillus Cereus*
6	+	rod	+	-	+	+	-	+	*Bacillus subtilis*
7	-	cocco bacilli	-	+	+	-	-	-	*Leuconostoc sp*
8	+	Spheri cal	+	-	+	+	-	+	*Micrococcus sp*

Key:

+ means positive

- Means negative

Table 3: Biochemical characteristics of isolates from African Locust beans from Leaves

Isolates	**Grams Rxn**	**Shape**	**Catalase**	**Indole**	**Vp**	**Oxidase**	**Methyl red**	**Citrate**	**Probable organisms**
1	-	rod	+	+	-	-	+	+	*Salmonella spp*
2	+	Cocci in cluster	+	+	-	+	+	+	*Staphylococcus Aureus*

3	+	Cocci in chains	-	-	-	+	+	-	*Streptococcus Lactis*
4	-	rod	+	-	-	+	+	+	*Pseudomonas aeruginosa*
5	+	Bacilli	-	-	-	-	-	-	*Lactobacillus plantarium*
6	+	rod	+	-	+	-	-	+	*Bacillus Cereus*
7	+	rod	+	-	+	+	-	+	*Bacillus subtilis*
8	-	cocco bacilli	-	+	+	-	-	-	*Leuconostoc sp*
9	-	rod	+	+	-	-	+	-	*Escherichia coli*
10	+	Spheri cal	+	-	+	+	-	+	*Micrococcus sp*

Key:

+ means positive

- Means negative

DISCUSSION

The suspected isolates recorded in table 1 includes *Salmonella* spp, *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus cereus* and *Bacillus subtilis*. *Salmonella* species has been reported to be transmitted through contaminated water and this bacterium is suspected carrier found in food handlers (Buker *et al.,* 2012). Bacillus species were the predominant microorganisms in this sample. The result of this study justifies the assertion of Ogbuonye (2018) that *Bacillus* species was the predominant bacteria involved in the fermentation of ogiri which is also a fermented soup condiment. *Pseudomonas* isolated in this sample is an aerobic organism and thrives well in the presence of oxygen and water (Ukaoma *et al.*, 2018). The locust bean seed usually comes with a substantial microbial local comprising spores of aerobic spore forming bacterial.

The result on table 2 shows the characteristics of isolates from those packaged with nylon. Suspected organisms include; *Salmonella spp Streptococcus lactis, Pseudomonas aeruginosa, Lactobacillus plantarium, Bacillus cereus, Bacillus subtilis, Leuconostoc sp and Micrococcus sp.* Eight isolates were isolated from the sample. *Bacillus spp.* which is a predominant organism obtained in this study and *Leuconostoc* spp. are capable of increasing the protein and fat contents of locust beans. This is similar to the study of Ogbadu and Okagbue (1988) who observed that various *Bacillus* species were responsible for the fermentation of African Locust Bean seeds. The result obtained from this study is similar to the work of Ihemeje *et al*., 2022 who also isolated *Lactobacillus, Pseudomonas* and *bacillus* from fermented melon seed.

The result from table 3 shows the biochemical characteristics of isolates from African Locust beans packaged with dry banana leaves. The suspected isolates are *Salmonella spp, Staphylococcus aureus, Streptococcus Lactis, Pseudomonas aeruginosa, Lactobacillus planetarium, Bacillus cereus, Bacillus subtilis, Leuconostoc sp, Escherichia coli* and

Micrococcus sp. The microorganisms isolated from locust beans packaged with dry banana leaves might be due to the contamination from leaves used as packaging materials. The leaves are usually picked up from the ground and on the farm land and are not normally washed thereby introducing different microorganisms from soil and insect droppings. Studies by (Antia and Ibrahim 2000) and (Odunfa 1983) has shown that several microorganisms are associated with locust bean fermentation. He also noted that the most abundant and the major dominant agent of fermentation after 24hours was *Bacillus subtilis*. They also noted the presence of *Leuconostoc mesenteroides* and *Staphylococcus* species after fermentation. Similar report also showed that *Bacillus subtilis*, *Leuconostoc mesenteroids* and *Staphylococcus* sp. were associated with the fermentation of African locust bean seeds to produce "iru" condiment (Omafuvbe *et al.*, 2002). However, the presence of *Escherichia coli* and S*taphylococcus aureus* are of public health concern and can lead to foodborne diseases. The microbes may be introduced into the products through the contaminated hands of handlers, unsterilized utensils, other materials being used for the processing and even from the air from the environment during the preparation of the product (Nwagu *et al* 2011; Tope, 2013). Achi (2005)

In conclusion, the results from this study shows that the use of plastic container as a packaging material should be encourage when compare to nylon and leaves for packaging of locust beans before consumption. Locust beans packaged with dry banana leaves harbors a lot of microorganisms that are able to cause foodborne diseases which are therefore of public health concern. It is therefore recommended that locust beans produced must be well preserved with the best packaging materials in order to prevent the entry and contamination by microorganisms

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