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Comparative Study of the Effect of Ginger Extract and Salt on the Microbial Load of Fermented African Locust Bean Seed (*Parkia biglobosa*).

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Abstract
This study was designed to access the comparative effect of ginger powder and salt on the microbial load of fermented African locust beans seeds (*Parkia biglobosa*). Microbial load in the fermented beans samples were determined at storage. The results showed that the locust bean seeds (plain) without any preservatives have high microbial load of ($6.8x10^5$)Cfu/g and ($6.5x10^5$) Cfu/g, ($8.5x10^5$) Cfu/g and ($7.2x10^5$)Cfu/g, ($8.8x10^5$) and ($7.4x10^5$) Cfu/g in week zero, 1 and 2 respectively with no growth of fungi. The locust bean seeds preserved with salt revealed TVC and TCC of $4.3x10^5$ and $2.4x10^5$Cfu/g in week zero, in week 1, TVC and TCC were $6.5x10^5$Cfu/g and $4.3x10^5$Cfu/g, while week 2 had TVC of $3.8x10^5$ and TCC were too numerous to be counted. The fermented African locust bean seeds preserved with ginger powder in week zero were TVC $5.2X10^5$ and TCC $4.8X10^5$ in week 1, the TVC and TCC were $3.3x10^5$Cfu/gand 5.2x105 while week 2 had Total Viable Count and Total Coliform Count were too numerous to be counted. In view of the results gotten above, addition of salt concentration to preserve *Parkia biglobossa* seed showed little microbial load count compared to plain locust bean seeds and locust bean seeds added with ginger powder. The result implied that salt is the best preservatives for locust bean seeds for longer shelf life and that if ginger extract will be used as preservatives, it should be added continuously to prevent the growth of numerous microbial count *Parkia biglobossa* seeds.

Keyword: Salt, Ginger powder, Locust bean seeds extract, Microbial load.

Introduction
African Locust bean tree (*Parkiabiglobosa)* is a perennial decidous tree which occurs in rainforest and arid zones of African countries. The tree has the capacity to withstand drought. It is primarily grown for tis pods that contain both the sweet pulp and valuable seeds. The seeds of *P. biglobosa* have been shown to contain up to 29% crude oil, 60% saccharose and also rich in vitamin C (Orwa *et al*., 2009).Aside being a good source of plant protein to man, it serves as a good source of protein for animal feeds (Obun, 2008). The quality and quantity of nutrient present in the food consumed by people in developing countries is very low compared to the actual requirement from balance diet for normal growth (FAO,2004). Rural dwellers in developing countries cannot afford animal produce which are rich sources of protein because they are either expensive or simply unavailable. This situation has made many people to depend on many carbohydrate diets; comprising cereal grains or starch root and tuber crop with low protein, thus leading to high level of malnutrition. In the quest of rural dweller to increase the protein level of their food, many wild fruits have been found to be good alternatives in many communities in South West Nigeria (Olujobi,2012).

Ginger (*Zingiber officinale*) is a rhizome, consumed as adelicacy, medicine, or spice. It lends its name to its genus and family (Zingiberaceae). Other notable

member of this plant family are Turmeric, Cardamom and Cinamom. Ginger production is a clusters of white and pink flower buds that bloom into yellow flowers. Ginger is often used as ingredient in making soup, as a spice in boiling water to make ginger tea. It can also be made into candy or used as flavouring for cookies, crackers and cake.

Salt (NaCl) is an ionic compound made of sodium and chloride ions. It has been found to be exceptionally important to humans for thousands of years, because it is one of the substances upon which all life activities depend on. All life need a supply of salt in order to survive. Salt ability to preserve food was a foundation of civilization which helped to eliminate the dependence on the seasonal availability of food. There have been two main sources of salt, sea water and the sodium chloride mineral halite (also known as rock salt). Because of the emergence of refrigeration, the need for salt as a preservative has decreased (He and Mac Gregor, 2001). However the taste is not the only reason for the continued use of sodium chloride in food, and sodium chloride still plays a role in reducing the growth of pathogens and spoilage microorganisms in foods.

Recently, the urgency for solution to the world food problem has thrown a challenge not only to the agriculturist and nutritionist but also to the foresters, to investigate the possibility of utilizing products from wild plants as additional source of protein, fat, vitamins, minerals and energy. Thus, study therefore aimed at investigating the effects of ginger powder and salt in the microbial load of fermented African locut beans sampled at storage.

Materials and Methods

This project work was carried out in Oyo State College of Agriculture and Technology, Igboora in Ibarapa Central Local Government Area. Raw locust bean seed, salt and natural preservatives (ginger) were bought at Towobowo local market in Igboora.

Processing of ginger: The ginger was rinsed thoroughly with tap water and oven dried at a 40^0C at room temperature for 48hours (2days), the preservative was milled to fine powder with the aid of blender, sieved, weighed and kept inside an air tight container until they were ready to be used.

Processing of African locust bean seeds: Processing of bean seeds was carried out at the Biology Laboratory, OYSCATECH., Igboora. Using the traditional method, raw locust bean seeds were boiled for 8 hours and left in the cooking pot overnight, cooking by boiling was repeated for another 4 hours the next day. Excess water was drained off using a sieve and the seeds were dehulled using a mortar and pestle, the dehulled shafts were separated off from the cotyledon by the use of sieve and washing with much water. The cotyledons were re-cooked for 40 minutes. The hot water was drained off and the cotyledons were spread in calabash lined with banana leaves covered with another calabash and wrapped with jute sacks. This was left in a cupboard in the laboratory to ferment for 72 hours (3 days) (Olujobi,2012).

Treating the processed beans:

The processed African locust bean seeds were divided into three portion. The first portion will serve as control and was labelled as sample A. The second portion

was mixed with ginger powder(100g locust beans with 10g of ginger) and labelled as sample B. The third portion was mixed with salt (100g locust beans with 10g of salt) which was labeled as sample C and all were replicated twice. Samples were collected fortnightly and evaluated for bacterial and fungal count.

Microbiological analysis: Total viable count (TVC), Total coliform count, and Fungi count were evaluated according to the method described by (Olusegun and Jacob, 2013).The locust beans samples were grinded with a sterile mortar and pestle, 1g of the sample was weighed into test tubes containing 9ml of sterile distilled water. Serial dilutions were made up to the 7th dilution. 28g of Nutrient agar powder was weighed in 1000ml distilled water, homogenized in water bath and autoclaved at 121^{o}C for 15 mins. The molten agar was employed and allowed to cool properly, while 0.5ml of the suspension in each tube was dispensed into sterile petri dishes and molten agar of about 9ml was added. This was also done for PDA(Potato dextrose agar) and MaCconkey Agar respectively. The plates were allowed to solidify, incubated at 37^{o}C for 24hrs for Nutrient agar and MacConkey agar, while PDA plates were incubated at 25^{0}C for 48 hours. After incubation, the organisms were counted for colony forming unit using colony counter. Sub-cultured on slant for further biochemical tests and identification were employed and stored in the refrigerator at 4^{0}C. The fungi were identified by using few drops of lactophenol cotton blue on a slide fungi picking were done with a sterile inoculating needle, teased on the slide and a cover slip was placed on it to avoid drying. It was then viewed under x10 objective lens. The fungi were identified by making use of the fungi Atlas (Monica, 2000).

Gram's Staining Techniques: Smear were made by putting a drop of sterile distilled water on the slide, a colony was picked from the culture of the test organisms and emulsify in a circular form. The slides were allowed to air dry and were then heat fix by passing the slides over the flame a couple of time. The slides were flooded with Crystal violet for 60sec and rinsed and then Gram's iodine for 60sec.It was rinsed and then flooded again with 70% ethanol to decolorize for 30sec after which Safranin was finally added for 60sec and rinsed. The slides were blot dried and then viewed under the microscope under oil immersion objective lens. Gram positive organisms showed purple while a Gram negative showed pink (Linnee, 2007).

Starch Hydrolysis Test: Starch agar plates were inoculated with the test organisms and incubated at 37^{0}C for 48hr.After incubation, the Starch agar plates were flooded with iodine and the results were observed (Linnee, 2007).

Sugar Fermentation Test: The organisms under test were inoculated in phenol red fermentation broth and incubated at 37^{0}C for 72hrs and results were observed for acid and gas production (Linnee, 2007).

Sulfur Indole Motility Test: A Sulfurn Indole Motility tube was inoculated with the organisms to be tested and incubated for 48hrs Kovac's reagent was add to the medium, result were observed. Pink/red color formation indicated indole formation while black precipitate in the medium indicated hydrogen sulfide production.

There were growth away from the stab line which indicated that the organisms were motile (Linnee, 2007).

Urease Test: Urea tubes were inoculated with the organisms under test and were incubated for 48hrs at 37^0C. A pink color formation indicated the breakdown of urea to ammonia and CO_2(Linnee, 2007).

MRVP Test: Inoculums of the test organisms were inoculated into MRVP broth and then incubated at 37^0C for 48hrs after which few drops of methyl red was added to the incubated medium. A red coloration indicated a positive test (Linnee, 2007).

VP (Vogesproskauer): The VP test was done from part of the medium for MR test after which three drops of 6% alpha napthol was added and followed by 0.2ml of 40% KOH. It was then agitated and allowed to stand for 30mins.A red colour indicated positive reaction (Linnee, 2007).

Catalase Test: This is use to differentiate those bacteria that produce the enzyme catalase such as Staphylococcus. A drop of hydrogen peroxide (H_20_2) was placed on a slide and a 24hr growth culture was emulsified on the slides. Presence of bubbles was observed as an indication of positive reaction (Linnee, 2007).

Citrate Test: An inoculum of the organisms under test were inoculated citrate medium (Simmon Citrate Agar) and incubated at 37^0C for 5days. A positive reaction presented a green colouration (Brenner and Stanleg, 2005).

Results

Microbial Load counts of fermented African Locust Bean Seeds

The plain (raw) African locust bean seeds which is the control showed Total viable count of $6.8x10^5$, no growth of fungi colony while Total Coliform count was 6.5x105. Fermented African locust bean seeds with salt revealed TVC of $4.3x10^5$and $2.4x10^5$ TCC with no growth for fungi. Fermented African locust bean seeds with ginger powder exhibit TVC to be $5.2x10^5$ and $4.8x10^5$for TCC with no growth of fungi colony in week 0 (Table 1). There were increment in the microbial load of plain African locust bean seeds in week 1 with TVC of $8.5x10^5$, TCC $7.2x10^5$, also no growth for fungi. More microbial load was shown in sample B with TVC of $6.5x10^5$ and TCC of $4.3x10^5$ which are high compared to week 0. Sample C showed less TVC $3.3x10^5$Cfu/g but increase TCC of Cfu/g respectively in week 1 (Table 2).

Plain African locust bean seeds showed highest TVC of $8.8x10^5$Cfu/g, $7.4x10^5$Cfu/g of TCC and no growth of fungi in week 2. Sample B showed low TVC of $3.8x10^5$ and many coliform which are too numerous to be counted. Fermented African locust bean seeds with ginger powder (C) revealed too numerous bacteria (coliform) which cannot be counted and Total viable count without growth of fungi (Table 3).

Isolation of Microorganisms:

A total of seven bacteria genera were isolated from various samples examined (Table 1-3). Further characterisation revealed the organisms to be *Escherichia coli, Staphylococcus aureus, Clostridium sp., Bacillus substilis, Bacillus sp. and Proteus vulgaris* (Table 4). *Escherichia coli*

Table 1:Total Viable Count of Microorganisms in the bean samples at week 0

Samples	Total Viable Count (TVC) Cfu/g	Total Fungi Count (TFC) Cfu/g	Total Coliform Count (TCC) Cfu/g
A (Raw Locust bean Control)	$6.8x10^5$	NG	$6.5x10^5$
B(Fermented African locust bean seeds with salt)	$4.3x10^5$	NG	$2.4x10^5$
C (fermented locust bean seeds with ginger powder)	$5.2x10^5$	NG	$4.8X10^5$

Key
NG- No Growth
100g of locust bean seeds plain without salt and ginger powder
100g of locust bean seeds with 10g of salt
100g of locust bean seeds with 10g of ginger powder

Table 2: Microbial load of isolates carried out on fermented locust bean seeds with salt, ginger powder and control for week 1

Samples	Total Viable Count (TVC) Cfu/g	Total Fungi Count (TFC) Cfu/g	Total Coliform Count (TCC) Cfu/g
A (Raw Locust bean Control)	$8.5x10^5$	NG	$7.2x10^5$
B(Fermented African locust bean seeds with salt)	$6.5x10^5$	NG	$4.3x10^5$
C (fermented locust bean seeds with ginger powder)	$3.3x10^5$	NG	$5.2X10^5$

Key
NG- No Growth
100g of locust bean seeds plain without salt and ginger powder
100g of locust bean seeds with 10g of salt
100g of locust bean seeds with 10g of ginger powder

Table 3: Microbial load of isolates carried out on fermented locust bean seeds with salt, ginger powder and control for week 2

Samples	Total Viable Count (TVC) Cfu/g	Total Fungi Count (TFC) Cfu/g	Total Coliform Count (TCC) Cfu/g
A (Raw Locust bean Control)	$8.8x10^5$	NG	$7.4x10^5$
B(Fermented African locust bean seeds with salt)	$3.8x10^5$	NG	TNTC
C (fermented locust bean seeds with ginger powder)	TNTC	NG	TNTC

Key
NG- No Growth
100g of locust bean seeds plain without salt and ginger powder
100g of locust bean seeds with 10g of salt
100g of locust bean seeds with 10g of ginger powder

Table 4: Biochemical Reaction pattern and Tests for identification of isolates microorganisms from the grouped African locust bean seeds

Test	Gram's reaction	Catalase	Coagulase	MR	VP	Urease	Citrate	Oxidase	Motility	Indole test	H_2S
A_1	-	+	-	-	-	-	-	-	-	+	-
A_2	+	+	+	+	+	+	+	-	-	-	-
A_3	+	-	-	-	-	-	-	-	+	-	+
B_1	+	-	-	-	+	-	+	-	+	-	-
B_2	-	+	-	+	-	+	-	-	+	+	+
C_1	+	+	+	+	+	-	+	+	-	-	-
C_2	-	+	-	+	-	+	+	-	+	-	+

Key
+- positive, -Negative,A-Control,B-Preservation with salt, C-Preservation with ginger powder, A1- Escherichia coli, A2- *Staphylococcus aureus,* A3- *Clostridium sp.,* B1- *Bacillus substilis,* B2- *Escherichia coli,* C1- *Bacillus sp.,* C2- *Proteus vulgaris*

showed negative reaction to all biochemical tests except catalase and Indole test reaction in control sample. *Staphylococcus aureus* showed positive result in all tests except in oxidase, motility, Indole test and H_2S reactions that was negative in control sample. *Clostridium sp.* Showed negative reaction to all the tests except being positive to Gram's reaction, motility and H_2S test for control sample. *Bacillus substilis* was negative in reactions to all tests but showed positive reaction to Gram's reaction, VP, Citrate and motility tests. *Escherichia coli* reacts positively with catalase MR, Urease, motility, Indole test and H_2S while negative reaction was exhibited with Gram's reaction, coagulase, VP, citrate and oxidase respectively for African locust bean seeds mixed with salt. Numerous *Bacillus sp.* were positively reacted with all the test except negative reactions urease, motility, Indole and H_2S tests, while proteus vulgaris showed positive reaction with catalase, MR, Urease, citrate, motility, H_2S and reacted negatively with Gram's reaction, coagulase, VP, Oxidase and Indole tests respectively for the locust beans seeds added to ginger powder (Table 4.)

Discussion

The increased number of colony count of raw African locust beans in week 0, 1 and 2 indicated rotten of the bean seeds compared to the sample A and B which is preserved with salt and ginger powder and may also be attributed to non preservation of the sample which allow multiplication of microorganisms. This result is in line with Adelekan and Nwadiuto (2012) who also reported high colony count of bacteria in raw locust bean ($4.0x10^3$) compared to fermented one ($1.2x10^1$, $1.28x10^5$ and $1.6x10^9$) respectively. Similar observation was observed by Ademola *et al.,* (2013) in their study of microbial load of Parkia big-lobossa seeds towards enhancing shelf life. The fermented African locust bean seeds preserved with salt showed low microbial load in week 0, 1 and 2 compared to the bean preserved with ginger powder. The result corroborated those of Ademola *et al.,* (2013) who observed least microbial load in the fermented locust bean seeds preserved with highest salt concentration.

The study also corresponds with that of Kolapo *et al.,* (2007) who reported reduction in bacteria population in meat preserved with salt. They also attributed it to anti-bacterial compounds present in salt. The fermented African locust bean seeds preserved with ginger powder that has high bacterial load may be due to low concentration of ginger powder added to the locust beans. The *staphylococcus aureus*, *E-coli* and *Clostridium sp.* encountered in the raw African locust bean seeds could be attributed to handling during its processing. This result is in line with the report of Wogu and Iwezeula (2013), who observed the aforementioned microbial load in ready-to-eat salad sold in Benin city and associated it with handling by cook and food handlers in the food centres. Similar report was made by Oni *et al.,*(2009) who reported *Staphylococcus aureus* as the leading cause of food poisoning. Udo *et al.,(*2009) also reported *Staphylococcus aureus* in high percentage in fresh vegetable salads in Calabar and the associated it to low level of standard in personal hygiene. Similar report of E-coli was also reported by Gbonjubola *et al.,* (2012) in vegetable salad sold in Zaria, Nigeria.

Bacillus sp., E-coli and *proteus vulgaris* observed in the fermentation of African locust bean seeds in this study could be attributed to moisture produced during storage and preservation by salt. This result is similar to the report of Odunfa (1981), Antai and Ibrahim (1986), Odunfa and Oyewole (1986), Diawara *et al.,* (1998), Ogueke and Aririatu (2004) where they all associated the presence of above microbial load to the cause of fermentation of *Parkia biglobossa*. Ogueke and Aririatu (2004) also reported *Proteus sp.* as one of the predominant sp. isolated throughout the period their study of microbial and organoleptic changes associated with Ugba. Omafuvb *et al.,* (2004) also reported *Bacillus substilis, Streptococcus sp.* as the associated microorganisms involved in the fermentation of *Parkia biglobossa* studied for chemical and biochemical changes associated with it. Similar report was made by Ogbadu and Okagbue (1998).

Conclusions

It is concluded from this study that, the African locust bean seeds preserved with salt has less microbial load while raw *Parkia biglobissa* had high microbial count, followed by the one preserved with ginger powder. Therefore, use of salt in locust bean seeds and other food items would decrease chance of food poisoning, reduce risk of food contamination and improve health status by using small quantity of salts. Salt can be relied upon to prolong the shelf life of locust bean seeds, since salt reduced the number of bacteria found in the sample preserved with salt.

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