

Preservation of Locust Bean Using Garlic and Ginger: Chemical and Microbial Changes

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ABSTRACT

This study aimed to determine the chemical and microbial changes in locust bean preserved using a combination of salt, garlic, and ginger. Fresh tomato locust bean was processed into condiment and divided into samples with varying concentrations of ginger, garlic, and salt (2.5% w/w). These samples were stored for 4 weeks, during which their proximate composition and microbial load were assessed weekly. The total viable count (TVC), lactic acid bacteria (LAB), and yeast counts were determined, and pure microbial isolates were identified. Results showed that samples containing 2.5% ginger and garlic exhibited increased levels of crude protein, crude fat, and ash content over the storage period. TVC and LAB counts in samples with garlic and ginger remained relatively stable compared to the control sample. Identified microorganisms included various species of *Lactobacillus*, *Leuconostoc*, and *Saccharomyces*, among others. The study concluded that the combination of garlic and ginger at 2.5% concentration effectively preserved locust beans for up to 4 weeks without deterioration.

Keywords: Locust bean, garlic, ginger, salt, biopreservative, microbial changes, chemical composition, shelf life, food preservation.

INTRODUCTION

The steep expense associated with animal protein has spurred interest in various leguminous seed proteins as potential sources of vegetable protein for both human consumption and livestock feed [1, 2, 3]. Legumes, among plant species, are recognized as primary sources of direct proteins [4, 5], particularly in regions such as developing and underdeveloped countries where access to animal protein may be limited due to economic, social, cultural, or religious factors [6]. African locust bean seeds are notably high in protein and are commonly fermented to create a flavorful food condiment known as dawadawa, which enhances the taste of soups and stews while also supplementing protein-deficient diets [7, 8, 9]. Among the leguminous plants utilized in several African countries is the African locust bean tree (*Parkia biglobosa*) [10, 11, 12]. The seeds are renowned for their role in producing a local condiment commonly referred to as Dadadawa (in Hausa) or Iru (in Yoruba). Moreover, *Parkia biglobosa* stands out for its exceptional protein quality, with its protein and amino acid composition well-documented [13, 14, 15]. Food preservation involves processes aimed at halting or significantly slowing down spoilage, which can be caused or accelerated by microorganisms [16, 17]. Preservation methods typically include inhibiting the growth of bacteria, fungi, and other microorganisms, as well as delaying the oxidation of fats that can lead to rancidity [18, 19, 20].

It also encompasses techniques to impede natural aging and discoloration that may occur during food preparation, such as enzymatic browning reactions [21, 22, 23, 24]. As highlighted by [25], one of the challenges facing traditional fermented foods is their limited shelf life. Therefore, fermentation must be halted or controlled after a certain period to prevent further microbial growth and spoilage. This can be achieved by preserving the seeds post-processing [25, 26, 27, 28]. 'Iru' typically has a shelf life of 2 to 3 days without additives, necessitating disposal within three days if not consumed. The bacteria, yeast, and molds responsible for deterioration require moisture for their metabolism, which must be completely eliminated to prevent their proliferation. To address the issues encountered by 'Iru' processors in prolonging the product's shelf life, the rate of deterioration will be investigated [29, 30, 31, 32]. Preservation techniques and preservatives serve to hinder or regulate the activities of microorganisms that cause food spoilage, a process also known as sanitization [33, 34, 35, 36]. These microorganisms, through their growth and metabolic processes, produce by-products that alter the texture, taste, flavor, and aroma of food. Preservatives are specifically formulated to counteract these changes, acting on both Gram-positive and Gram-negative spoilage organisms [36]. The incorporation of preservatives in food products aims to prolong shelf life

and prevent spoilage [37, 38, 39, 40]. Extended shelf life enhances marketability, as products remain fresher for longer periods, providing consumers with more time to use them [23, 41, 42, 43, 44, 45]. Salt preservation, a traditional method practiced globally, involves maintaining a salt concentration of 6-10% in tissues, inhibiting the growth of most spoilage bacteria [56]. This preservation method can be applied through dry salting, brine salting, injection salting, or a combination thereof, with dry salting being the most widely utilized technique, particularly in the processing of salted fish. Biopreservation entails the use of natural compounds to extend the storage life and improve the safety of foods [58]. Ginger, derived from the rhizome of *Zingiber officinale*, is a widely utilized member of the ginger

family (*Zingiberaceae*). Besides its medicinal value, ginger is frequently incorporated into beverages for its preservative properties due to its antimicrobial activity [37]. Similarly, garlic, renowned for its antimicrobial effects against both Gram-positive and Gram-negative bacteria, also offers nutritional benefits and exhibits antioxidant potential [43]. Both ginger and garlic are universally accepted, cost-effective, and well-tolerated ingredients. While chemical preservatives remain popular for microbial control in food, there is growing interest in exploring organic alternatives. This study focuses on utilizing organic chemical preservatives, specifically ginger and garlic, to enhance product shelf-life stability while maintaining its distinctive sensory attributes.

Aim and Objectives of Research

This study aimed to analyze the chemical and microbial properties of locust bean preserved with salt, garlic, and ginger. The specific objectives were as follows:

To establish the optimal fermentation conditions for converting 'Iru,' a protein-based condiment.

To assess the nutritional and microbiological impacts of varying salt concentrations on processed Iru (*Parkia biolobosa*).

To investigate the nutritional and microbiological effects of different ginger concentrations on processed Iru (*Parkia biolobosa*).

To examine the nutritional and microbiological impacts of various garlic concentrations on processed Iru (*Parkia biolobosa*).

To determine the most effective preservative for processed "Iru" (*Parkia biglobosa*).

MATERIALS AND METHODS

African locust bean, garlic, ginger, and salt were purchased from different local markets in Anambra State. The materials were sorted, cleaned, and kept in high-density polyethylene to avoid moisture uptake and contamination before use.

Experimental design

The experiment was a mixture design obtained using Design Expert 12. The experiment had a total of 9 runs. The design key is shown in Table 1.

Table 1

	A Component 1	B Component 2	C Component 3
Total run	Salt (%)	Garlic (%)	Ginger (%)
1	1.0	0	0
2	0	1.0	0
3	0	0	1.0
4	1.5	0	0
5	0	0	1.5
6	0	1.5	0
7	2.5	0	0
8	0	2.5	0
9	0	0	2.5

Ginger flour

The ginger bulbs were first sorted and cleaned, then peeled and sliced into smaller pieces. The sliced ginger was soaked in sodium bicarbonate for 10 minutes and

then oven dried for 30 minutes at 60°C. After which it was milled using a disc attrition mill and sieved afterwards with a 2mm sieve.

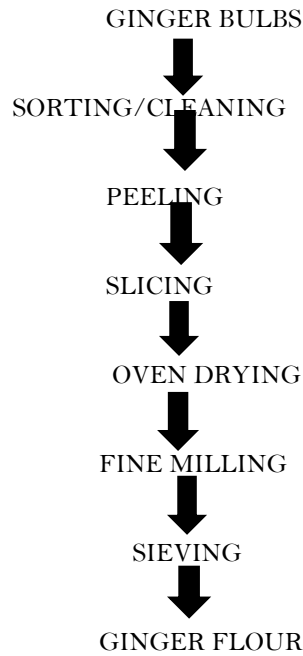


Figure 1: FLOW CHART FOR GINGER FLOUR
Garlic flour

The garlic bulbs were first sorted and cleaned, then peeled and sliced into smaller pieces. The sliced ginger was soaked in sodium bicarbonate for 10 minutes and

then oven-dried for 30 minutes at 60°C. Afterward, it was milled using a disc attrition mill and sieved with a 2mm sieve.

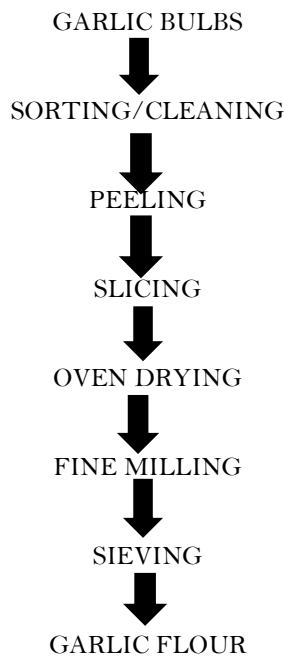


Figure 2: FLOW CHART FOR GARLIC FLOUR

PRODUCTION OF LOCUST BEAN

The seeds were boiled for 10 to 12 hours, dehulled to separate the cotyledons from the hulls. The dehulled cotyledons were boiled in little water for about 10 minutes, drained through a raffia basket. They were allowed to cool and were covered with special leaves, and

allowed to ferment for 6 days under the sun. The fermented seeds were ground or pounded to form a dark paste and molded into balls or desired shapes. They were dried under the sun and stored in an airtight container for use. This process is shown below:

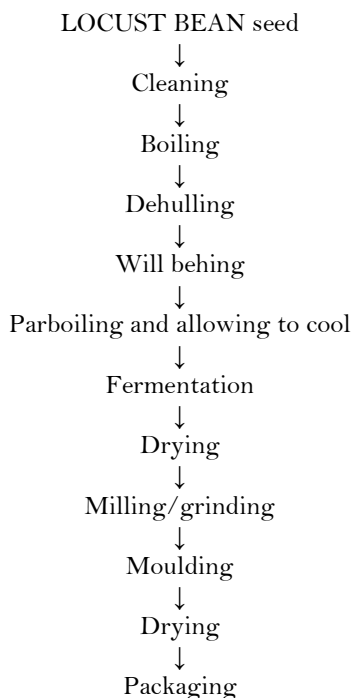


Figure 3: FLOW CHART FOR IRU CHEMICAL ANALYSIS Proximate and Mineral analyses

Proximate and Mineral analyses were carried out using Official Methods of Analysis by Association of Analytical Chemists [15].

Microbial and Biochemical Tests

Microbial and Biochemical Tests analyses were carried out using methods of [26]. The data underwent statistical analysis using analysis of variance (ANOVA),

and means were distinguished using the least significant difference (LSD) method, employing Statistical Package for Social Sciences (SPSS) version 20.0.

RESULTS AND DISCUSSION

Proximate Composition

Table 2 presents the Proximate Composition of locust bean with salt, Garlic, and Ginger as Bio-preservatives on a dry weight basis. Addition of ginger, garlic, or their blends decreased the crude protein content in the preserved locust bean compared to the control sample (Table 3). However, all locust bean samples showed a significant ($p < 0.05$) increase in crude protein content with prolonged storage. In the absence of any biopreservatives, the initial protein content of 24.41%

rose to 25.18%, representing a 27.46% increase during storage. Conversely, samples with 2.5% garlic and 2.5% ginger exhibited percentage increments of 23.31% and 24.35% over the initial protein content, respectively. Notably, higher increases in crude protein contents were observed in samples preserved with ginger powder. The addition of salt did not yield similarly high increases in protein content. Throughout the storage period, the crude fat contents of all samples increased significantly

compared to the control sample. Samples containing 2.5% garlic and 2.5% ginger, and 2.5% salt exhibited crude fat content ranges of 0.5-1.36%, 0.77-1.46%, and 0.78-1.42%, respectively, while the control sample varied from 0.37-0.74%. This increase in fat content could be attributed to the proliferation of microbes during storage, as microbes in high numbers tend to augment the protein and fat content of the food they inhabit. The presence of ginger, garlic, and salt inhibited microbial growth, consequently limiting the quantity of protein and fat added to the locust bean. Furthermore, the presence of essential and fatty oils in garlic and ginger may have contributed to the observed increase in fat content of the biopreserved locust bean samples. The

crude fiber content of all samples decreased over the storage period, with locust bean exhibiting significantly ($p < 0.05$) higher crude fiber content than the other biopreserved samples. Ash content increased with storage time, with samples containing ginger, garlic, or salt showing increases in the range of 172-234.1%, while the control sample experienced a 165% increase. During storage, the carbohydrate content of locust bean, and samples containing 2.5% ginger and 2.5% garlic were significantly ($p < 0.05$) lower compared to other samples. This reduction in carbohydrate and fiber levels could be attributed to microbial utilization, particularly of reducing sugars, which are easily metabolized by microorganisms.

Table 2: Proximate Composition of locust bean with salt, Garlic and Ginger as Biopreservatives (dry weight basis).

Time in weeks	Sample code	% Crude Protein	% Crude Fat	% Crude Fibre	% Crude Ash	% CHO
0	2.5% Salt	24.10±0.03c	0.50±0.01d	1.05±0.01a	1.39±0.01a	72.96±0.01c
	2.5% Garlic	23.39±0.03d	0.77±0.01d	0.94±0.01a	1.29±0.01c	73.61±0.01c
	2.5% Ginger	24.27±0.02a	0.78±0.01a	1.25±0.01a	1.46±0.01b	72.24±0.01b
1	2.5% Salt	24.14±0.01c	1.19±0.01c	0.98±0.01b	2.85±0.02c	70.84±0.15b
	2.5% Garlic	24.27±0.01c	1.20±0.01c	0.81±0.01a	3.22±0.02b	70.50±0.15b
	2.5% Ginger	24.27±0.01a	1.25±0.01a	0.91±0.01a	3.36±0.02c	70.21±0.15ab
2	2.5% Salt	24.20±0.01c	1.29±0.02b	0.85±0.01c	4.00±0.01b	69.66±0.01c
	2.5% Garlic	24.44±0.01b	1.39±0.02b	0.88±0.01a	4.27±0.01a	69.02±0.01a
	2.5% Ginger	24.34±0.01a	1.05±0.02b	0.85±0.01a	4.20±0.01b	69.56±0.01a
3	2.5% Salt	24.33±0.01b	1.36±0.01a	0.68±0.01e	4.10±0.01a	69.53±0.01b
	2.5% Garlic	24.58±0.01b	1.39±0.01ab	0.78±0.01a	4.27±0.01a	68.98±0.01a
	2.5% Ginger	24.64±0.01a	1.22±0.01a	0.84±0.01a	4.26±0.01a	69.04±0.02a
4	2.5% Salt	24.64±0.03a	1.36±0.01a	0.77±0.01d	4.10±0.01a	69.13±0.01b
	2.5% Garlic	24.75±0.03a	1.46±0.01a	0.68±0.01a	4.31±0.01a	68.80±0.01a
	2.5% Ginger	24.75±0.03a	1.42±0.01a	0.81±0.01a	4.31±0.01a	69.71±0.01a

*Values are mean scores± Standard deviation of triplicate

*Data in the same column bearing different superscript differ significantly (p < 0.05)

Microbial Load of Biopreserved locust bean during Storage

Table 3 presents the Microbial Load of Biopreserved locust bean during Storage. Throughout the storage period, the Total Viable Count (TVC), Lactic Acid Bacteria (LAB) count, and yeast and mould count increased in all samples. In the locust bean sample without any biopreservative, the TVC increased from 3.41 to 13.45 log cfu/g from week 2 to week 4 of storage. Conversely, the locust bean sample containing 2.5% ginger exhibited a TVC ranging from 3.34 to 6.07 log cfu/g during the initial 4 weeks of storage. Samples containing 2.5% garlic showed no observable growth during the first 4 weeks of storage, effectively suppressing TVC. Furthermore, locust bean samples containing 2.5% salt maintained a TVC lower than 5.0

log cfu/g throughout storage. Table 4 illustrates the LAB counts in locust bean samples, reflecting a similar increasing trend as TVC over the 4-week storage period. After 4 weeks, samples with 2.5% garlic and 2.5% ginger exhibited the lowest LAB levels, while samples with 2.5% salt showed LAB counts comparable to the control sample. Regarding yeast count (Table 5), the control sample and samples preserved with 2.5% ginger exhibited counts ranging from 13.67 to 14.99 log cfu/g after 4 weeks of storage. Locust bean samples preserved with 2.5% garlic showed yeast counts ranging from 3.82 to 4.60 log cfu/g, while those preserved with 2.5% salt displayed the highest yeast counts ranging from 4.82 to 5.80 log cfu/g after 4 weeks of storage.

TABLE 3 TOTAL VIABLE COUNT OF BIOPRESERVED LOCUST BEAN IN STORAGE (CFU/G)

Sample	Week 0	Week 1	Week 2	Week 3	Week 4
LOCUST BEAN	0.00 ± 0.00 ^A	5.79 ± 0.24 ^C	6.40 ± 0.21 ^D	7.40 ± 0.13 ^D	8.54 ± 0.25 ^E
LOCUST BEAN + 2.5% ginger	0.00 ± 0.00 ^A	3.82 ± 0.13 ^B	5.30 ± 0.25 ^C	6.93 ± 0.13 ^C	8.65 ± 0.31 ^E
LOCUST BEAN 2.5% SALT	0.00 ± 0.00 ^A	3.71 ± 0.15 ^B	6.43 ± 0.21 ^D	6.98 ± 0.13 ^C	7.99 ± 0.23 ^D
LOCUST BEAN 2.5% garlic	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$)

TABLE 4. LACTIC ACID BACTERIA COUNT OF BIOPRESERVED LOCUST BEAN IN STORAGE (CFU/G)

Sample	Week 0	Week 1	Week 2	Week 3	Week 4
LOCUST BEAN	0.00 ± 0.00 ^A	5.79 ± 0.24 ^C	6.40 ± 0.21 ^D	7.40 ± 0.13 ^D	8.54 ± 0.25 ^E
LOCUST BEAN + 2.5% ginger	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	3.52 ± 0.18 ^B	3.57 ± 0.13 ^B	4.40 ± 0.22 ^C
LOCUST BEAN 2.5% SALT	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	3.39 ± 0.13 ^B	3.54 ± 0.13 ^B	3.64 ± 0.23 ^B
LOCUST BEAN 2.5% garlic	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A	0.00 ± 0.00 ^A

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$)

TABLE 5: YEAST COUNT OF BIOPRESERVED LOCUST BEAN IN STORAGE (CFU/G)

Sample	Week 0	Week 1	Week 2	Week 3	Week 4
LOCUST BEAN	0.00 ± 0.00 ^A	4.77 ± 0.22 ^C	5.65 ± 0.19 ^C	7.92 ± 0.34 ^D	8.91 ± 0.11 ^C
LOCUST BEAN + 2.5% ginger	0.00 ± 0.00 ^A	4.51 ± 0.19 ^C	5.78 ± 0.23 ^C	7.62 ± 0.29 ^D	8.93 ± 0.24 ^C
LOCUST BEAN 2.5% SALT	0.00 ± 0.00 ^A	5.32 ± 0.18 ^D	6.32 ± 0.20 ^D	6.68 ± 0.18 ^C	9.69 ± 0.23 ^D
LOCUST BEAN 2.5% garlic	0.00 ± 0.00 ^A	3.65 ± 0.15 ^B	3.59 ± 0.22 ^B	3.62 ± 0.17 ^B	3.64 ± 0.11 ^B

Samples with same letter are not significantly different for the assessed parameter ($P < 0.05$)

Identity of Bacteria Isolated in Biopreserved Locust bean Samples

The bacterial strains found in the locust bean samples treated with salt, garlic, and ginger, as outlined in Table 6, were determined to be gram-positive, non-spore-forming, and catalase-negative. Analysis of biochemical tests and identification keys revealed the presence of *Lactobacillus brevis*, *Lactobacillus plantarum*, *Lactobacillus delbrueckii*, *Lactobacillus*

fermentum, *Lactobacillus acidophilus*, and *Leuconostoc mesenteroides*, as detailed in Table 6. Additionally, yeasts isolated from the locust bean samples were identified as *Saccharomyces cerevisiae*, *Saccharomyces lactis*, *Hansenula anomala*, *Rhodotorula glutinis*, *Rhodotorula flava*, and *Rhodotorula rubra*, as presented in Table 6.

Table 6. Morphological and Biochemical Characteristics of Bacteria Isolates From Locust Beans

Test	Isolates					
	B1	B2	B3	B4	B5	B6
Morphology	Rods	Rods	Cocci	Rods	Rods	Rods
Color of growth	Cream	Cream	Cream	Cream	Cream	Cream
Gram reaction	+	+	+	+	+	+
Catalase test	-	-	-	-	-	-
Growth at:						
15°C	+	+		-	-	-
45°C	-	-		+	+	+
Production of CO ₂	-	+	+	-	+	
Glucose	+	+	+	-	+	-
Lactose	+	-	-	-	+	+
Arabinose	+	+	-	-	+	-
Trehalose	+	-	+	+	+	+
Salicin	+	-	-	-	-	+
Galactose	+	+	-	-	+	+
Sucrose	+	+	-	+	+	+
Raffinose	+	+	-	-	-	+
Probable identity of organism	<i>Lactobacillus plantarum</i>	<i>Lactobacillus brevis</i>	<i>Leuconostoc mesenteroides</i>	<i>Lactobacillus delbrueckii</i>	<i>Lactobacillus fermentum</i>	<i>Lactobacillus acidophilus</i>

CONCLUSION AND RECOMMENDATIONS

These studies have shown that salt, garlic and ginger added at 2.5% w/w could be used as effective biopreservatives in locust bean for not less than 4 weeks. The results obtained in this study showed that the crude protein, fat, and ash content increased with storage. Although, only the combination of garlic and ginger as biopreservatives was effective in reducing bacteria and yeast counts. Garlic at 2.5% alone were more effective against LAB and yeast load than water

at the same concentration. Locust bean treated with 2.5% ginger was effectively pre-served against microbial deterioration, lowered chemical characteristics and was even found acceptable to the tasters. In some countries in Africa, where power is in limited supply, the addition of 2.5% garlic and or ginger to locust beans can be of assistance in enhancing its shelf life particularly when locust bean is in season.

Recommendations

The food industry should explore the rich nutritional potential and beneficial functional properties of this legume for the development and formulation of innovative food products. Utilizing ukpo in weaning formulas, especially when fermented due to its high protein content, could be instrumental in combating

protein-energy malnutrition in children. Encouraging the adoption of advanced techniques in the production of locally fermented foods is essential. Careful handling and proper cleaning of processing materials/equipment such as pots, grinders, mortar and pestles, etc., are crucial to prevent microbial

contamination. Additionally, covering the seed flour adequately is recommended. The findings of this study should be integrated into Nutrition Education Programs and disseminated to various stakeholders including food industries, households, communities, and health centers (including antenatal sessions).

This dissemination will facilitate the effective utilization of this legume.

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