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Microbiological and nutritional quality of optimized African Locust Beans using *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 As Starter culture

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Abstract
This study assessed the microbiological and nutritional qualities of locust beans produced, using *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 as a Starter Culture. African locust beans seeds were purchased from Imiegba market, Nigeria. Two hundred grams (200g) of the beans were boiled at 121℃ for 4 hours, dehaulled and subsequently cooked at 121℃ for 2h. Fifty grams of the cotyledon was inoculated with 5mL of *L. plantarum* SAL-IMIE02, *L. fermentum* SAL-IMIE01 and (1:1) *L. planetarium* SAL-IMIE02 and *L. fermentum* SAL-IMIE01, and incubated at 37℃ for 3days in a fermenting jar. Microbiological analysis, proximate composition, vitamin C, mineral composition, and pH was carried out using the standard laboratory methods. Results analysis revealed that *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 locust beans showed no detectable growth (0.0×10^3 cfu/g) for fungi, Enterobacteriaceae, *Pseudomonas* spp., coliforms, and *Salmonella/Shigella* spp. while the control exhibited high microbial load 1.0×10^3 - 9.6×10^3 cfu/g. Control sample contained a significantly high moisture content (16.5 ± 0.2%), compared to the optimized formulations (10.5 ± 0.1%). Crude protein (41.2 ± 0.3%), crude fat (18.7 ± 0.2%) content was significantly enhanced in the optimized formulations compared to the commercial locust beans (30.3 ± 0.2%; 15.3 ± 0.2%) observed for the control. Crude fiber was highest (7.1 ± 0.2%) in *L. fermentum* SAL-IMIE01 locust beans whereas carbohydrate content (38.5 ± 0.2%) and ash (4.5 ± 0.2%) was higher in control. Potassium and sodium were significantly reduced in fermented with *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 locust beans. Similarly, *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 improved locust beans calcium, iron, and zinc bioavailability. *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 should be formulated into commercial starter culture and adequately publicized for standardization of traditional production methods.

Keywords: locust beans, microbiological safety, nutritional qualities

Introduction

African locust beans are a traditional condiment produced from the seeds of *Parkia biglobosa* tree, a leguminous plant native to Africa. The tree belongs to the family Fabaceae and is a member of the *Parkia* species (Adeoye *et al*., 2018) [5]. It is a multipurpose tree, grown for its pods containing sweet yellow pulp and valuable seeds. It serves as a nutritious non-meat protein substitute in rural settings, although it is widely used as condiments and flavouring agents in soups and sauces (Adejumo *et al*., 2013) [2].

Commercial locust beans are produced on a traditional small-scale in household under highly variable conditions (Ibeabuchi *et al*., 2014) [17]. The fermentation process of the traditional *iru* production is initiated by a diverse community of naturally occurring microorganisms introduced through multiple routes, including the seed surface, processing utensils, ambient environment, and human handling, and is accomplished by the natural temperatures of the tropics (Adelekan and Nwadiuto, 2012) [3].

Previous study on the traditional Africa locust beans fermentation revealed *Bacillus subtilis* as the dominant bacteria (Obafemi *et al*., 2022; Owusu-Kwarteng *et al.,* 2022; Adeniyi *et al.,* 2024) [4, 22], other associated bacteria include *Bacillus licheniformis, Bacillus megaterium,* and *Leuconostoc mesenteriodes* (Agunwah *et al.,* 2024) [7]. Their biochemical activities help develop the desirable taste, smell, appearance, and texture that make these foods acceptable and enjoyable (Akpi *et al*., 2020; Owusu-Kwarteng *et al*., 2022) [9].

However, microbial hazards may be introduced from processing utensils, unhygienic environment, and human handling leading to variation in the microbial profile of each batch (Agunwah *et al.,* 2024) [7]. In addition to the uncontrolled nature of the traditional production process of African locust beans, inadequate awareness and use of hygienic practices further compromise the microbiological quality, safety and shelf life of this indigenous fermented foods (Anyogu *et al.,* 2021) [10]. This highlights the need for improved hygiene practices, and development of optimized production process, particularly the development of starter cultures to ensure safety and consistency.

Lactic acid bacteria (LAB) are an important group of probiotic microorganisms found in fermented foods, and

they are generally considered safe. LAB is a diverse group of Gram-positive bacteria that are facultative anaerobes, non-spore-forming, non-motile, and tolerant to acid. They can appear as single cells or form couples, tetrads, or long chains. These bacteria have common metabolism and physiology, and they primarily ferment sugars into lactic acid (Makut *et al*., 2022) [19]. Lactic acid bacteria, such as the *Limosilactobacillus fermentum* and *Lactiplantibacillus plantarum* have been documented to produce metabolites such as bacteriocins and exopolysaccharides and excellent antioxidant and antimicrobial properties with good probiotics potential (Salmat Musah *et al.,* 2022). Therefore, this study was design to assess the microbiological and nutritional qualities of locust beans produced, using *Lactiplantibacillus plantarum*SAL-IMIE02 and *Limosilactobacillus Fermentum* SAL-IMIE01as a Starter Culture.

Materials and Methods

StudyArea

Collection of Materials

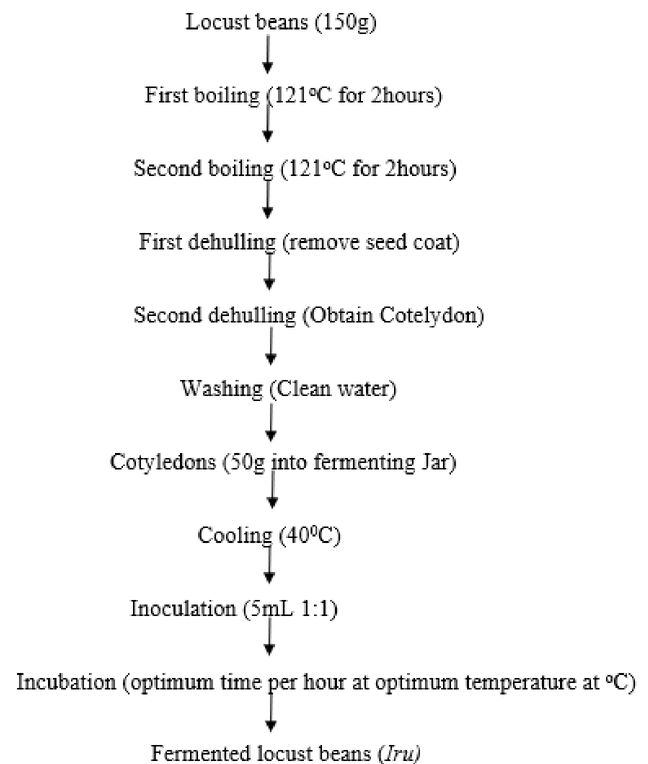
The locust beans seed and traditionally fermented locust beans was purchased from Imiegba main market Edo State, Nigeria. The fermented locust beans were used as control while the locust beans were identified by a botanist in the Department of Science and Biotechnology, Nasarawa State University, Keffi, Nasarawa state. Lyophilized *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 which were previously isolated, characterized, identified and Preserved by Salmat Salisu Musah during master's research work, was used as a starter culture. Other equipment used includes a cabinet dryer, pH meter, weighing balance, mortar, pestle and microscope and analytical grade chemicals from the Department of Microbiology Laboratory, Nasarawa State University, and Keffi.

Preparation of Inoculum Starter Culture

The LAB strains used as inoculum (*L. plantarum* CIP 103151 and *L. fermentum* CIP 102980 1:1) were prepared by transferring a loopful of an overnight culture from MRS agar into 10 mL MRS broth each and incubated at 37°C for 24 h. One hundred microliters of the 24-hour-old culture was transferred into 10 mL MRS broth each and incubated at 37°C for 16 h (overnight). 5ml of each of the starter cultures was used in the production of the optimized fermented locust beans (Iru) (Akabanda *et al*., 2014).

Optimization Production of Iru

One and fifty Hundred grams (150g) of African locust beans was boiled in a pressure pot for 2 hours at 121°C to soften the firmly attached seed coasts and further boiled for another 2 hours at 121°C. Excess water was drained off and the seeds were dehaulled by slightly pounding the seeds with a sterile metal pestle and mortar. Further removal of the seed coat was achieved by gently smashing seed to obtain the the cotyledons with a sterile metal pestle and mortar then was washed with clean water. The cotyledons were again cooked at 121°C for 1 hour, the water was drained off and 50 g of the cotyledons was then poured into two (2) fermenting containers respectively (sterile glass jars) and allowed to cool to 40°C before inoculation. The two jars of 50g cotyledons were then inoculated with 5mL starter culture (*Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 1:1) respectively at 40°C and incubated at 37°C for three (3) days with slight modification to the method of Ibeabuchi *et al*. (2014) [17]



Production of fermented locust beans (*Iru*)
Source: Ibeabuchi *et al*. (2014) [17]

Microbiological Examination of Locust Beans From Optimization Production And Traditional Produced Sample

Locust beans from both optimization production methods and traditional production methods were examined for their viable bacterial count, enteric bacteria, and fungi. Tryptic Soy Agar (TSA) was used to determine the viable count of bacteria, while Sabouraud Dextrose Agar (SDA) was employed for the cultivation of fungi and yeasts. MacConkey Agar was utilized to isolate Enterobacteriaceae. Cetrimide Agar (CA) was used to isolate Pseudomonas species, and Mannitol Salt Agar (MSA) was employed to identify Staphylococcus species. Eosin Methylene Blue Agar (EMB) was used to detect coliforms. Additionally, Xylose Lysine Deoxycholate Agar (XLD) was utilized for the potential isolation of Salmonella and Shigella.

The pour plate method of Cheesbrough (2000) was used for microbial count. Serial dilution was carried out using normal saline to 10^{-6} dilution, and 1 ml of 10^{-3} diluent was transferred to a petri dish. All media were added into separate plates, swirled gently and allowed to solidify and incubated at 37°C for 48 hours. The numbers of colonies were counted on the plates, taking into consideration the dilution factor to obtain the total viable count and the enteric bacteria. While yeasts and moulds were determined by inoculating an aliquot of 1ml of the sample on Sabouraud Dextrose Agar, the plates were incubated at 25°C for 72 hours. The number of colonies was counted and expressed as colony-forming units per gram (cfu/g) for both samples.

Microbiological quality of fermented locust beans (*Iru*)
The optimized sample (M) showed **no detectable growth** (0.0×10^3 cfu/g) on Sabouraud Dextrose Agar (SDA), MacConkey Agar (MCA), Cetrimide Agar (CA), Eosin Methylene Blue (EMB), and Xylose Lysine Deoxycholate (XLD) agars, indicating the absence of fungi, Enterobacteriaceae, *Pseudomonas* spp., coliforms, and *Salmonella/Shigella* spp., respectively. However, microbial growth was observed on Mannitol Salt Agar (MSA) at 5.2×10^4 cfu/g. The plate on Tryptic Soy Agar (TSA) for the optimized sample was reported as TFTC (Too Few To Count). In contrast, the commercial sample (C) exhibited a higher microbial load. The TSA plate was too numerous to count) where varied growth was observed on SDA (4.0×10^3 cfu/g), CA (1.0×10^3 cfu/g), and MSA (9.6×10^3 cfu/g). However, no growth was recorded on MCA, EMB, and XLD agars.

Total Microbial Count of the Optimized and Commercial Locust Beans

Samples	TSA	SDA	MCA	CA	MSA	EMB	XLD
M	TFTC	$0.0{\times}10^3$	$0.0{\times}10^3$	$0.0{\times}10^3$	$5.2{\times}10^4$	$0.0{\times}10^3$	$0.0{\times}10^3$
C	TNTC	$4.0{\times}10^3$	$0.0{\times}10^3$	$1.0{\times}10^3$	$9.6{\times}10^3$	$0.0{\times}10^3$	$0.0{\times}10^3$

Key: M: Fermented optimized locust beans; C: Commercial locust beans; TSA: Tryptic Soy Agar (general bacteria); SDA: Sabouraud Dextrose Agar (fungi/yeast); MCA: MacConkey Agar (Enterobacteriaceae); CA: Cetrimide Agar (Pseudomonas); MSA: Mannitol Salt Agar (Staphylococcus spp.); EMB: Eosin Methylene Blue Agar (Coliforms); XLD: Xylose Lysine Deoxycholate Agar (Salmonella/Shigella); TNTC: Too Numerous to Count; TFTC: Too Few to Count.

Determination of Proximate Composition of Samples
Proximate composition of fermented locust beans, samples were determined. Five commercial samples were use. This is to ensure choice of best commercial sample to be used as control. The seeds were grounded to powder form by using a blender. The powder samples were sieve to obtain uniform size that was analyzed for moisture, protein, fat, ash, fiber and nitrogen free extract by the methods of Association of Analytical Chemist (AOAC, 2003).

Determination of Moisture Content
Moisture content is a crucial factor in food quality, preservation, and shelf-life determination. It affects the stability and microbial growth of food products. The moisture content was determined using the oven-drying method as follows:

Apparatus and Materials Used
Analytical balance, Desiccator, Crucible with lid, thermosetting oven, and Tongs.
Clean crucibles were dried in a hot air oven at 105°C for 1 hour to ensure complete dryness. The crucibles were cooled in a desiccator for 30 minutes, then weighed and recorded as W_1. A 5 g of each sample was placed into the crucible and weighed as W_2. The crucibles with each of the samples were oven-dried at 105°C for 4 hours until a constant weight was obtained. The dried crucibles were cooled in a desiccator for 45 minutes and weighed again as W_3. The formula below was used to calculate the % Moisture Content;

$$\% \text{ Moisture Content} = \frac{W2 - W3}{W2 - W1} \times 100$$

W_1 = Weight of empty crucible
W_2 = Weight of crucible + sample before drying
W_3 = Weight of crucible + sample after drying (constant weight)
% Total Solid = 100 − % Moisture Content

Determination of Ash Content
The ash content represents the total mineral content in the sample, indicating inorganic residue left after complete combustion.

Apparatus and Materials Used
Muffle furnace, Analytical balance, Crucibles, Desiccator and Tongs.
A clean crucible was dried at 100°C for 30 minutes in a hot air oven. The crucible was transferred to a desiccator, cooled for 30 minutes, and weighed as W_1. A 5 g of each sample was placed into the crucible and reweighed as W_2. The sample was placed inside a muffle furnace at 550°C for 4 hours. After ashing (white ash residue was obtained), the sample was cooled in a desiccator for 1 hour and weighed as W_3. The formula below was used to calculate the % Ash Content:

$$\% \text{ Ash Content} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where:
W_1 = Weight of empty crucible
W_2 = Weight of crucible + sample before ashing
W_3 = Weight of crucible + sample after ashing
% Organic Matter = 100 −% Ash Content

Determination of Crude Fat Content
The crude fat content was determined using the Soxhlet extraction method with petroleum ether (40-60°C boiling range) as the solvent.

Apparatus and Materials Used
Soxhlet extraction apparatus, Petroleum ether, Thimbles, Analytical balance, heating mantle and Round-bottom flask
A clean thimble was dried and weighed as W_1. A 5 g of each sample was weighed into the thimble and reweighed as W_2. A clean, dry 500 ml round-bottom flask was weighed as W_3. The Soxhlet extractor was assembled, with the thimble placed inside the extraction chamber and the round-bottom flask filled halfway with petroleum ether. The apparatus was placed on a heating mantle at 34°C and allowed to siphon for 5 hours. The thimble was removed, and the petroleum ether was evaporated. The flask with extracted oil was oven-dried at 100°C for 5 minutes and cooled in a desiccator before reweighing as W_4. The formula below was used to calculate the % Crude Fat:

$$\% \text{ Crude Fat Content} = \frac{W3 - W1}{W2 - W1} \times 100$$

Where:
W_1 = Weight of empty thimble
W_2 = Weight of thimble + sample before extraction
W_3 = Weight of empty flask
W_4 = Weight of flask + extracted oil

Determination of Crude Fibre Content

Crude fiber content was determined by sequential acid and alkali digestion, followed by ashing.

Apparatus and Materials Used

Beakers (250 ml), Muslin cloth, Buckner funnel, Muffle furnace, Analytical balance.

A 3 g of each of the sample was weighed as W_1 into a 250 ml beaker containing 200 ml of 1.25% sulfuric acid (H_2SO_4). The mixture was heated at 85°C for 2 hours and then cooled. The solution was filtered through muslin cloth and washed with hot distilled water. The residue was transferred to another beaker containing 200 ml of 1.25% potassium hydroxide (KOH) and heated for 2 hours. The residue was washed, dried at 120°C, and weighed as W_2. The dried sample was ashed at 550°C for 30 minutes, cooled, and weighed as W_3. The formula below was used to calculate % Crude Fibre;

$$\% \text{ Crude Fibre Content} = \frac{W2 - W3}{W1} \times 100$$

Where:
W_1 = Initial weight of sample before extraction
W_2 = Weight of the oven dried residue after digestion
W_3 = Weight of the residue after ashing

Determination of Crude Protein Content (Kjeldahl Method)

A 1 g of sample was digested with 12 ml H_2SO_4 and $CuSO_4$-K_2SO_4 catalyst at 420°C for 30 minutes. The digest was diluted with 80 ml distilled water and 25 ml NaOH and distilled into 5 ml boric acid with methyl red indicator. The distillate was titrated with HCl until the endpoint (colour change to dark yellow). The formula below was used to calculate % Nitrogen;

$$\% \, Nitrogen \;=\; (V2|V1) \times C \times 0.0140 \times (S - B) \times 100$$

$$\% \, Protein = \% \, Nitrogen \times 6.25$$

Where;
V1 = Volume of sample solution used for titration (mL)
V2 = Volume of titrant (HCL) used during titration (mL)
C = Concentration of the acid (HCL) used for titration (mol/L)
0.0140 = constant representing molecular weight of nitrogen in grams per millimole
S = Volume of acid used for titration of the sample (mL)
B = Volume of acid used for titration of the Blank (mL)
6.25 = conversion factor from nitrogen to protein (proteins contain 16% of Nitrogen)

Determination of Carbohydrate Content

The carbohydrate content of the samples was determined by difference, meaning it was calculated as the difference between 100% and the sum of moisture, protein, fat, fiber, and ash content. The moisture content, crude protein, crude fat, crude fiber, and ash content were determined separately using standard procedures.

The percentage carbohydrate content for each sample was calculated using the formula:

$$\% \, Carbohydra = 100 - (\% \, Moisture + \% \, Fat + \%Protein + \%Fibre + \%Ash)$$

Determination of pH

The pH of the samples was measured using a digital pH meter. The pH level indicates the acidity or alkalinity of the sample, which is essential for food stability and microbial activity.

Apparatus and Materials Used:

pH meter, Distilled water, Beaker (50 mL), Magnetic stirrer, Glass electrode, Buffer solutions (pH 4.0 and pH 7.0).

The pH meter was calibrated using standard buffer solutions (pH 4.0 and 7.0). A 5 g of each sample was weighed and mixed with 50 mL of distilled water in a beaker. The mixture was stirred for 5 minutes using a magnetic stirrer to ensure proper dissolution. The pH electrode was inserted into the mixture, and the pH reading was recorded. The measurement was repeated three times and recorded for each sample.

Determination of Vitamin C (Ascorbic Acid) Content

Vitamin C content was determined using the 2,6-Dichlorophenolindophenol (DCPIP) titration method, which is based on the reduction of DCPIP by ascorbic acid.

Apparatus and Materials Used:

Burette, Pipette, Conical flask (250 mL), Standard DCPIP solution (0.1 mg/mL), freshly prepared ascorbic acid standard solution, Blender, Filter paper and Distilled water.

A 5 g for each sample was measured and blended with 50 mL of distilled water for 2 minutes. The mixture was filtered using Whatman No. 1 filter paper, and the filtrate was collected. A 10 mL of standard ascorbic acid solution was placed in a conical flask. The DCPIP solution was filled in a burette. The DCPIP solution was titrated against the ascorbic acid solution until the blue color disappeared. The volume of DCPIP used was recorded. A 10 mL of the sample filtrate was placed in a conical flask. The DCPIP solution was titrated into the sample extract until a persistent light pink color appeared. The volume of DCPIP used was recorded. The Vitamin C content for each sample was calculated using the formula:

$$Vitamin \; C \; \left(\frac{mg}{100mL}\right) = \frac{T \times S}{V}$$

Where:
T = Volume of DCPIP used for sample (mL)

S = Concentration of standard ascorbic acid (mg/mL)
V = Volume of sample used (mL)
The analysis was performed in triplicates and recorded.

Mineral Quantification Procedure

The concentrations of calcium (Ca), potassium (K), sodium (Na), magnesium (Mg), iron (Fe), and zinc (Zn) in the samples were determined using Atomic Absorption Spectrophotometry (AAS) and Flame Photometry, following standard procedures outlined by AOAC (2012) [12].

Sample Preparation and Digestion

The samples were first oven-dried at 105°C to achieve a constant weight. They were then finely ground using a laboratory mill to ensure homogeneity. 1.0 g of the ground sample was accurately weighed and placed into a 250 mL digestion flask. A mixture of concentrated nitric acid (HNO) and perchloric acid ($HClO_4$) in a 1:1 ratio (10 mL total) was added to the flask. The digestion process was carried out on a hot plate at 120–150°C until a clear solution was obtained, indicating complete digestion of organic matter. After cooling, the digested solution was filtered through Whatman filter paper and diluted to 50 mL with deionized water for analysis.

Determination of Calcium (Ca), Magnesium (Mg), Iron (Fe), and Zinc (Zn) Using AAS

The concentrations of Ca, Mg, Fe, and Zn were measured using an Atomic Absorption Spectrophotometer (Buck Model 20A, Buck Scientific, USA) at their respective element-specific wavelengths. The AAS was first calibrated using certified standard solutions prepared from 1000 mg/L stock solutions, appropriately diluted for each element. A blank solution (deionized water) was used to zero the instrument before sample measurements.
For calcium, the wavelength was set at 422.7 nm, while magnesium was measured at 285.2 nm. Iron was analyzed at 248.3 nm, and zinc at 213.9 nm. The digested samples were aspirated into the air-acetylene flame of the AAS, and the absorbance values recorded were used to determine the mineral concentrations by interpolating from standard calibration curves.

Determination of Sodium (Na) and Potassium (K) Using Flame Photometry

Sodium and potassium were analyzed using a Flame Photometer (e.g., Jenway PFP7 or equivalent model). The instrument was calibrated using standard solutions prepared from 1000 mg/L stock solutions, with sodium standards diluted to 0, 5, 10, 20, and 50 mg/L and potassium standards to 0, 2, 5, 10, and 20 mg/L.
The flame photometer was set to detect sodium at 589.0 nm and potassium at 766.5 nm, using a propane-butane-air flame for excitation. The sample solutions were aspirated into the flame, and the emission intensities were recorded. The sodium and potassium concentrations were determined by comparing the emission values to those of the calibration standards.

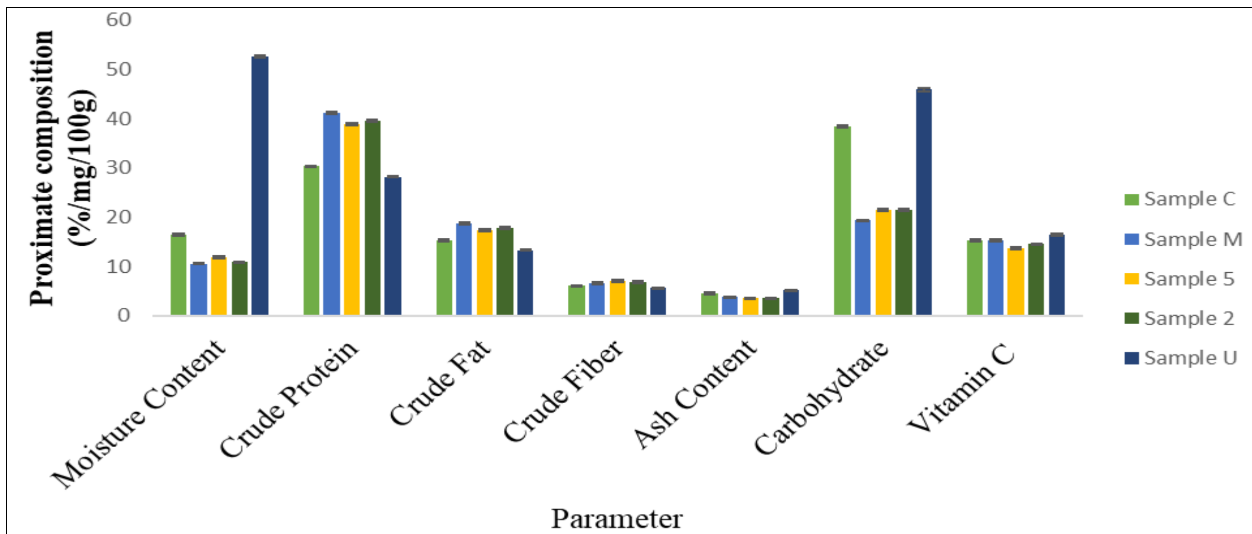
Total microbial count of the optimized and commercial locust beans

Samples	TSA	SDA	MCA	CA	MSA	EMB	XLD
M	TFTC	0.0×10^3	0.0×10^3	0.0×10^3	5.2×10^4	0.0×10^3	0.0×10^3
C	TNTC	4.0×10^3	0.0×10^3	1.0×10^3	9.6×10^3	0.0×10^3	0.0×10^3

Key: M: Fermented optimized locust beans; C: Commercial locust beans; TSA: Tryptic Soy Agar; SDA: Sabouraud Dextrose Agar; MCA: MacConkey Agar; CA: Cetrimide Agar; MSA: Mannitol Salt Agar; EMB: Eosin Methylene Blue Agar; XLD: Xylose Lysine Deoxycholate Agar; TNTC: Too Numerous to Count; TFTC: Too Few to Count.

Proximate content, and Vitamin C Content of Samples

presents the proximate composition, and vitamin C content of five locust bean samples. Non-fermented locust beans contained a significantly high moisture content (21.60 ± 0.2%), compared to the optimized formulations, Sample M (10.5 ± 0.1%), Sample 2 (10.9 ± 0.1%) and Sample 5 (11.8 ± 0.2ᵃ%). The crude protein content was significantly enhanced in the optimized formulations compared to the commercial locust beans and the non-fermented locust beans. Locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 recorded the highest value (41.2 ± 0.3%), followed closely by *L. plantarum* 103150 (39.5 ± 0.2%) and *L. fermentum* SAL-IMIE01 (38.9 ± 0.2%) locust beans.
Similarly, crude fat content was also significantly higher in the optimized samples chiefly in *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 locust beans (18.7 ± 0.2%), followed by *L. plantarum* SAL-IMIE02 (17.8 ± 0.2%) and *L. fermentum* SAL-IMIE01 (17.3 ± 0.2%) compared to 15.3 ± 0.2% and 13.33± 0.2% observed for control samples. In terms of the crude fiber content, locust beans fermented with *Limosilactobacillus fermentum* SAL-IMIE01 had the highest value (7.1 ± 0.2%). However, ash content was higher in the commercial sample (4.5 ± 0.2%) compared to the formulated samples 3.5% to 3.8% respectively.
The carbohydrate content was observed to be inversely proportional to the protein and fat content. Commercial locust beans had the highest carbohydrate content (27.4 ± 0.2%), while the locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01recorded a significantly lower value (19.3 ± 0.2%). Lastly, the vitamin C value was slightly lower in the optimized samples ranging from 13.7 to 15.2 mg/100g compared to 15.3 ± 0.2 mg/100g observed for commercial sample.



Key: Sample C (commercial locust beans); Sample M (locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01); and Samples 2 and 5 (locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 respectively); Sample U (Non-fermented locust beans)

Fig 1: Proximate composition of samples collected

pH Values of the optimized and commercial locust beans samples

Table 4.0 shows the pH values of the various locust bean samples. Samples commercial locust beans and locust beans fermented with *L. plantarum* SAL-IMIE02 had a similar pH values of 6.2 which is significantly lower compared to locust beans produced with both the *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01. Overall, the non-fermented samples recorded a significant lower pH value of 5.9.

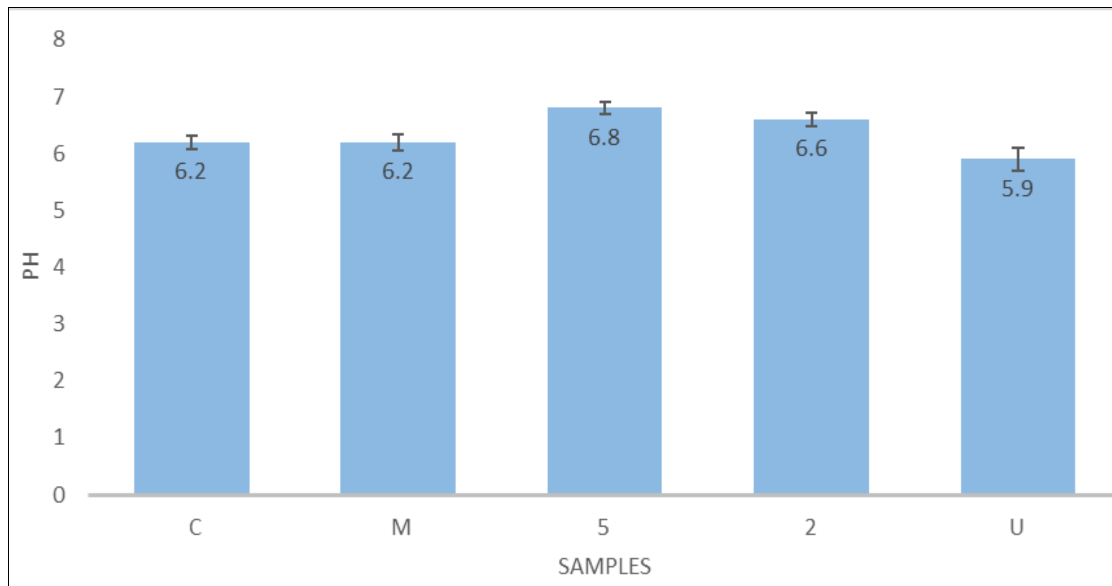


Fig 2: pH levels of samples collected

Key: Sample C (commercial locust beans); Sample M (locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01); and Samples 2 and 5 (locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 respectively); Sample U (Non-fermented locust beans)

Mineral Composition of Sample C, U and M

Table 4.1 presents the mineral composition of commercial and the optimized locust beans. Potassium (K) and sodium (Na) levels were significantly higher in commercial locust beans (18.5 ± 0.25 mg/100g and 2.9 ± 0.1 mg/100g, respectively) compared to locust beans fermented with *L. plantarum* SAL-IMIE02 SAL-IMIE02 and *L. fermentum* SAL-IMIE01 (15.8 ± 0.3 mg/100g and 2.6 ± 0.1 mg/100g). Similarly, magnesium (Mg) was also higher in the commercial and non-fermented sample (4.3 ± 0.2 and 17.20 ± 0.1 mg/100g) than in the optimized sample (3.7 ± 0.3 mg/100g). On the contrary, calcium (Ca), zinc (Zn), and iron (Fe) were significantly elevated in locust beans fermented with *L. plantarum* SAL-IMIE02 and *L. fermentum* CIP SAL-IMIE02.

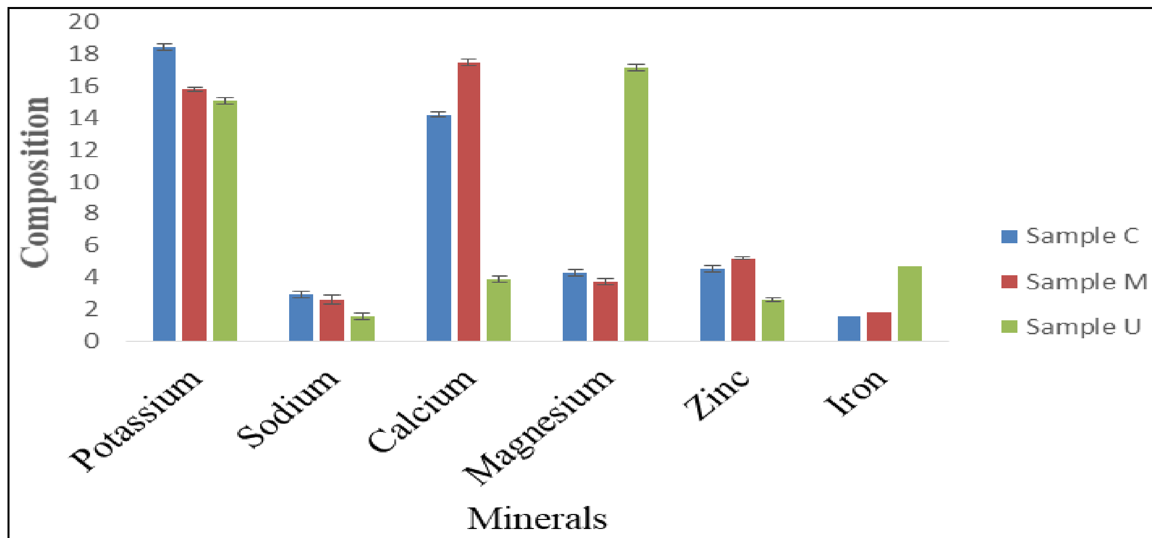


Fig 3: Mineral compositions of samples collected

Key: Sample C (commercial locust beans); Sample M (locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01); and Samples 2 and 5 (locust beans fermented with *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 respectively); Sample U (Non-fermented locust beans).

Discussion of Results

Locust beans (*Parkia biglobosa* seeds) fermentation is popular all-over West Africa countries, and it is often carried out under an uncontrolled condition with little or no hygiene. This, no doubt can compromise the microbiological quality, safety and nutritional composition. This study therefore investigated the use of probiotic *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 for the production of locust beans with improved consistency, safety, and nutritional profile. Lactic acid bacteria are well known for their ability to secrete antimicrobial compound, enhance food quality via breakdown of antinutritional factors as well as for the biosynthesis of essential nutrients (Lopez *et al*., 2000; Kahala *et al*., 2023) [18].

The microbiological evaluation of the optimized locust beans sample (M) fermented with *L. plantarum* and *L. fermentum* starter cultures revealed a significantly improved microbiological safety compared to the commercial sample (C). The absence of detectable microbial growth on selective and differential media such as Sabouraud Dextrose Agar (SDA), MacConkey Agar (MCA), Cetrimide Agar (CA), Eosin Methylene Blue (EMB), and Xylose Lysine Deoxycholate (XLD) for sample M shows possible inhibitory effect of the selected lactic acid bacteria strains employed as starter cultures. This observation also suggests, their potential competitive exclusion of both spoilage and pathogenic microbes. This may be as a result of acidification and production of antimicrobial metabolites such as bacteriocins and hydrogen peroxide (Zhang *et al*., 2018; Du *et al*., 2022) [15, 26].

In contrast, the commercial sample (C) had too numerous to count growth on tryptic soya agar plate. This suggest probable poor hygiene of fermentation process, and/or the intrinsic inconsistency which often accomplish natural fermentation and poor handling in the production process. The presence of these bacteria possess risk to consumer health. The observed too few to count colonies on trptic soya broth for optimized samples supports the efficacy of the starter culture (*L. plantarum* strain SAL-IMIE02 and *L. fermentum* SAL-IMIE01) in reducing microbial contaminant (Afolake *et al*., 2018; Anyogu *et al*., 2022) [6].

The use of selected probiotic strains as starter cultures led to significant improvement in protein (41.2 %), fat (18.7 %), and fiber content (7.1 %) (Table 4.3). The reduction in moisture content in the optimized samples possibly indicates reduced water composition. This is required for longer shelf life due to reduced microbial growth. Similarly, high protein of the optimized sample reflects the metabolic effect of LAB starter culture through fermentation, this will no doubt improve protein assimilation and digestibility of the product. More so, the increased crude fat and fiber may be attributed to lactobacillus enzymatic potential, hydrolyzing the bound in the lipids and carbohydrate component of *iru*. Ogunbanwo *et al*. (2014) reported that fermentation is often accomplished by increased protein and fat content, hence the finding of this study is comparable with previous study. However, Olukomaiya *et al*. (2020) reported insignificant spike in the moisture and a decrease in protein during natural fermentation of locust beans, possibly as a result of microbial degradation. The use of probiotic strains such *L. plantarum* and *L. fermentum* therefore offers targeted nutritive enhancements leading to probiotic health benefits. Remarkably, though vitamin C content was somewhat reduced, however it is within the acceptable nutritional limits. The observed decrease may be due to partial degradation during or role in the fermentation process (Atere *et al*., 2019) [13].

The pH values of the samples show some variability, with locust beans fermented with *L. plantarum* SAL-IMIE02 having the highest pH (6.9), followed by locust beans fermented with *L. fermentum* SAL-IMIE01. Commercial and *L. plantarum*-fermented samples both have a similar pH of 6.2. The pH differences between locust beans fermented with *L. plantarum* SAL-IMIE02 and the other samples could potentially influence the stability and processing characteristics of the samples, as pH can affect the texture, flavour, and shelf-life of food products (Yilmaz *et al*., 2022) [25].

Similar low pH values (6.2) observed in both the commercial and *L. plantarum*-fermented samples indicate

acidity that accompany lactic acid fermentation and may affect the locust beans preservation by inhibiting the growth of pathogenic and spoilage bacteria and it also improves sensory qualities such as tartness. The higher pH observed for locust beans fermented with *L. plantarum* SAL-IMIE02 and *L.* SAL-IMIE01) may be due to negative microbial interaction or competition between the two strains. It may also be due to the buffering capabilities of the by-products (proteins and peptides) resulting from the fermentation by these strains (Azokpota *et al*., 2005).

Potassium and Sodium were significantly reduced in the locust beans fermented with *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE01 compared to the commercial sample. Potassium is an important nutrient for muscle function and fluid balance, while sodium plays a vital role in nerve transmission and electrolyte regulation (Olukomaiya *et al*., 2020). The reduction in potassium and sodium in the optimized sample corroborates the findings of Olukomaiya *et al*., (2020), who opined that mineral leaching during processing of the locust beans prior fermentation or by fermenting microbes' activities could reduce these minerals.

Similar to the findings of Adegoke *et al*. (2010) [1], optimization of the locust beans improves calcium, iron, and zinc bioavailability in the present study. Calcium, zinc and iron were significantly high in optimized sample (17.53 mg/100g; 5.2 ± 0.3 mg/100g; 1.8 ± 0.2 mg/100g) compared to the commercial samples (14.23 mg/100g; 4.5 ± 0.2 mg/100g; 1.5 ± 0.1 mg/100g. This implies a potential health benefit of the product particularly improve bone health, muscle function, immune function, enzyme activity, and hemoglobin formation the enhanced calcium content might be due to enzymatic breakdown of anti-nutritional factors like oxalates and phytates by *Lactilantibacillus* spp, which can release bounded minerals during fermentation. However, magnesium significantly reduced in optimized sample (3.7 mg/100g) compared to the commercial sample (4.3 mg/100g). This may not be unconnected to microbial consumption or binding during fermentation.

Conclusion

The present study achieved optimized fermentation of African locust bean (Iru) with probiotic starter culture of *L. plantarum* SAL-IMIE02 and *L. fermentum* SAL-IMIE02. The use of these probiotic strains considerably enhanced the microbiological quality of *iru,* as optimized samples showed no detectable growth (0.0×10^3 cfu/g) for fungi, Enterobacteriaceae, *Pseudomonas* spp., coliforms, and *Salmonella/Shigella* spp.

Nutritional quality assessment revealed that the optimized *iru* showed significantly high crude protein (41.2 ± 0.3%), crude fat (18.7 ± 0.2%), and crude fiber (7.1 ± 0.2%). Similarly, the mineral composition of the optimized samples indicated improved availability of calcium (14.23 ± 0.45 mg/100g), zinc (4.5 ± 0.2 mg/100g), and iron (1.5 ± 0.1 mg/100g), although potassium and sodium levels were lower compared to commercial products.

Recommendations

1. Large-scale production of *iru* using *Lactiplantibacillus plantarum* SAL-IMIE02 and *Limosilactobacillus fermentum* SAL-IMIE01 is encouraged to boost the economic potentials of the product.
2. Owing the potential of the two probiotic strains, commercial starter culture formulations should be developed and publicized to create awareness amongst local processors, with the ultimate of standardizing traditional methods of production.
3. Local producer should be trained on hygiene and aseptic techniques that will ensure the microbiological safety and wide acceptability of Africa originated fermented food

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