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Evaluation of the Effects of B*acillus* Species Co-culture on some Quality Characteristics of Fermented African Locust Beans (*Iru*)

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ABSTRACT

Fermentation of African locust beans (*Parkia biglobosa)* is primarily facilitated by *Bacillus* species, producing a condiment known by various names across Nigeria (*Iru* in South-Western Nigeria). This work studied the potential of two starter cultures and their coculture for the fermentation of African locust bean (*Parkia biglobosa*) to produce *Ir*u. Single cultures of *Bacillus subtilis* (BS) and *Bacillus licheniformis* (BL) and their coculture (BSBL) were used as starter cultures for fermentation at 37^oC for 72h. Fermenting African locust beans were analyzed for microbial counts and pH during fermentation while proximate analysis and sensory evaluation were carried out following fermentation.. Counts increased from initial inoculum sizes of 0.5 McFarland standard ($1.5x10^8$ Cfu/g) where sample BL showed the highest count at 24h (11.3log$_{10}$ Cfu/g) and at 72h (15.1log$_{10}$ Cfu/g). Sample BSBL had the lowest counts at 24h (10.4log$_{10}$ Cfu/g) and at 72h (14.3log$_{10}$ Cfu/g). Proximate analysis showed significant differences ($P< 0.05$) with sample BS having the highest crude protein (8.8%) and moisture (69.4%) contents while sample BL yielded highest ash content (0.8%).Statistical analysis showed significant differences in results of sensory evaluation ($P< 0.05$) with coculture fermented *Iru* receiving the highest ratings for aroma, taste, colour, and overall acceptability whereas products fermented with single cultures were less preferred. Findings suggest that single cultures of *B.subtilis* and *B.licheniformis* enhance the nutritional quality of *Iru*, while their coculture produce a more acceptable product. Further studies are recommended to assess safety characteristics with a view to promoting industrial applications to enhance consistency in quality and safety.

Keywords: African locust beans, Iru fermentation, Bacillus subtilis, Bacillus licheniformis, Coculture, Acceptability

INTRODUCTION

Fermentation is the chemical transformation of organic matter through microbial metabolism, facilitated by a diverse array of enzymes (Chai *et al.*, 2021) where organic substrates are converted into simpler compounds (Adesanya *et al.*, 2021). Fermentation, recognized as one of the oldest and most cost-effective biotechnological processes, serves not only as a natural method for food preservation and the development of novel food products but also contributes significantly to the human diet by transforming raw substrates into value-added products with enhanced nutritional quality and improved sensory attributes (Das *et al.*,2019).

Microbial fermentation could occur spontaneously through the activities of naturally occurring organisms or natural flora of substrates or through intentional inoculation with starter cultures. In Spontaneous fermentation, which implies a fermentation process that occurs without the intentional and direct addition of a microbial starter by the human hand that depends on naturally occurring microorganisms (Capozzi *et al.*, 2020), relies on indigenous microbial populations, such as bacteria, yeasts, and molds, which metabolize available nutrients and produce desirable changes in food, beverages, or other substrates. This type of fermentation has challenges such as inconsistency, potential spoilage, and contamination risks (Toor *et al.*, 2022). Controlled fermentation, on the other hand, can occur with various types of tools such as temperature control, regulated oxygen deprivation, and the addition of supplementary microorganisms. (Zhang *et al.*, 2021). Controlled fermentation involves the careful and deliberate management of regulating factors such as temperature, oxygen levels, and the specific microorganisms involved (Atfaoui *et al.*, 2021).

African locust bean is found in a wide range of environments in Africa and is primarily grown for its pods that contain both a sweet pulp and valuable seeds. The nutritional significance of African locust beans lies in their rich composition of proteins, high calorific value, essential amino acids and fatty acids content, vitamin, and fiber (Ajiboye and Hammed, 2020). The alkaline fermentation of *Parkia biglobosa*, primarily facilitated by *Bacillus* species, is a traditional method yielding a nutritionally significant condiment, with scientific investigations elucidating microbial dynamics, biochemical transformations, and nutritional outcomes. Traditionally, the seeds of African locust beans are allowed to undergo a fermentation process that transforms them into condiments known by various names across the region. These include *Ogiri- igala* (South-East and South-South Nigeria), *Soumbala* (Mali, Côte d'Ivoire, Guinea, and Burkina Faso), *Dawadawa* (Ghana, Niger, and Northern Nigeria), and *Iru* (South-Western Nigeria) (Sanya *et al.*, 2013). The fermentation of African locust beans is mostly facilitated by bacteria, particularly *Bacillus* species such as B*acillus subtilis*, *Bacillus pumilus*, and *Bacillus licheniformis*. Bacillus species are aerobic, rod-shaped Gram-positive spore forming bacteria common in soil and water (Tamang *et al.*, 2020). These species are used in many industrial, agricultural, pharmaceutical, and medical processes. Studies on the microbiology of the fermentation of African locust bean seeds have identified *Bacillus* species as the primary microorganisms responsible for the fermentation process, contributing significantly to protein hydrolysis and flavor development (Tersoo-Abiem *et al.*, 2021; Adesanya *et al.*, 2021). The predominant species is *Bacillus subtilis*, but other species like *Bacillus pumilus*, *Bacillus megaterium*, and *Bacillus licheniformis* can also be found (Diawara *et al., 1992*; N'Dir *et al.*, 1997).

The challenge of utilizing controlled fermentation in traditional settings lies in balancing the benefits of safety and consistency (Bogueva & Danova, 2024). With the necessity of maintaining the distinct sensory and quality characteristics that drive consumer acceptability. To address this challenge, , this study aims to evaluate the effects of *Bacillus* species cocultures isolated and purified from retail I*ru,* on some key quality characteristics of fermented African locust beans, thereby establishing whether selected strains can replicate or enhance the traditional profile under controlled conditions. Ultimately, the outcomes of this research will provide a vital foundation for developing standardized starter cultures and optimized protocols for large-scale iru production, paving the way for the industrialization of traditional African condiments without sacrificing the quality consumer expect.

MATERIALS AND METHODS

Study Area

Samples of spontaneously fermented *Iru* were bought from some markets situated in Abuja Municipal Area Council (AMAC) in the Federal Capital Territory (FCT), Abuja Nigeria. . Abuja, which is centrally located within Nigeria, lies between latitudes $8^0$25N and $9^0$20N and longititudes $6^0$45E and $7^0$39E. The FCT has a population of over 3 million people and serves as major administrative, commercial and cultural center in Nigeria, inhabited by people from diverse ethnic and cultural backgrounds (Ayobolu, 2025). With an average annual rainfal of 1,100 to 1600 mm, the FCT has a tropical savanna climate with rainy and dry seasons stretching from April to October and November to March respectively (Nigerian Meteorological Agency, 2023). A host of markets in strategic spots across AMAC provide for the needs of its diverse ethnic groups. Markets selected for sampling of retail *Iru* in this study include those situated in Garki, Jabi, Bambilla, Nyanya, Kurudu , Kabusa, Lokogoma and Gaduwa.

Sample Collection

A total of 15 fermented *Iru* samples were purchased from 15 different retailers across the selected markets. Samples were placed in sterile plastic bags and transported on ice packs to Baze University for further analysis within 24h of collection.

Isolation and Characterization of *Bacillus* Species from Retail Fermented *Iru*

Preparation of Food Homogenate

Retail *Iru* samples were made into food homogenates in a 1:9 ratio of samples in sterile water respectively. These homogenates were then heated to 80^0C for 15 min to destroy vegetative cells/non spore formers. Heat treated homogenates were then subjected to tenfold serial dilutions (to 10^{-6}). Exactly 0.1ml of appropriate dilutions of each sample was spread onto sterile Nutrient agar plates, and incubated at 37 ˚C for 24-48 h until the appearance of distinct colonies. Distinct colonies from each plate were selected and subcultured onto fresh Nutrient agar plates to obtain pure cultures (Olaniran *et al*, 2020).

Identification and Characterization of Presumptive Bacillus Species

Isolates were subjected to morphological, biochemical, and molecular characterization for identification. Morphological identification involved examining colonial morphology and cell structure with the aid of a light microscope. Isolates were Gram stained following which Gram -positive isolates were subjected to biochemical tests and molecular characterization using 16s rRNA sequencing and Basic Local Alignment Search Tool (BLAST). The pure cultures were preserved by storing them in Nutrient Agar slants at 4ºC until used (Muigg *et al.,*2022).

Production of Fermented African Locust Bean using *Bacillus* Species Starter Cultures obtained from Retail Iru

Preparation of African locust bean seeds for fermentation was done using the method of Olaniran *et al.* (2020). The African locust beans seeds were sorted manually to remove dirt and other unwanted material, followed by soaking in water for 10 h to de-pulp the seeds. Pulp-free seeds were then boiled in a pressure cooker at 128kpa for 5h. The seeds were then dehulled manually to remove the seed coats. Dehulled seeds were washed multiple times in a wide bowl under running water to separate the seeds from the pulp, and further boiled for 3h to soften the seeds, then allowed to cool to room temperature.

Inoculation and Fermentation of African Locust Bean

The inoculation and fermentation of African locust bean seeds were done as described below

Standardization of Inoculum

Two confirmed *Bacillus* species isolates *(B. Subtilis* and *B. Licheniformis*) obtained from retail *Iru* samples (Garki 2 and Jabi 4) in this study were selected (Table 2) for use as starter cultures for laboratory *Iru* production. Isolates were standardized using the procedure of Caroll *et al.* (2020) ; each pure isolate was sub-cultured on Nutrient Agar and incubated at 37 °C for 24h. Colonies were picked with a sterile loop and transferred into test tubes containing sterile saline to make McFarland standard 0.5 (approximately $1.5x10^8$ CFU/g) of cells. For coculture starter cultures, standardized inoculums (0.5 McFarland standards) of *Bacillus subtilis* and *Bacillus licheniformis* were further diluted by equal volumes of sterile saline to obtain approximately half the number of cells ($0.75x10^7$CFU/ml)

Inoculation and Incubation of African Locust Beans with Standardized Bacillus Species Inoculum

Boiled and cooled unfermented African locusts were divided into 3 samples of 300g each. Samples were each inoculated with 1ml of a *Bacillus* culture as shown in Table 1 and incubated at 37°C for 72h

Table 1: Starter Cultures in Laboratory Production of Fermented African Locust Beans

African Locust Bean Sample Code	**Starter Culture**	**Incubation Temperature (°C)**	**Fermentation Period (hours)**
BS (Control A)	*Bacillus subtilis* only (Garki 2)	37	72
BL (Control B)	*Bacillus licheniformis* only (Jabi 4)	37	72
BSBL (Challenge sample)	*B. subtilis* + *B. licheniformis*	37	72

Determination of the Proximate Composition of African locust Bean Fermented Using Cultures of *Bacillus* Species Obtained from Retail *Iru*

Proximate composition analysis of the unfermented African locust bean, and fermented locust bean was carried out using the method of Pedrazzani *et al.*, (2024). Samples were analyzed for crude protein, crude lipid, ash content, carbohydrate, crude fiber, and moisture content.

Acceptability of African Locust Bean Produced using Cultures of Bacillus Species obtained from Retail Iru

Sensory evaluation was carried out using twenty-Five semi-trained panelists to assess the sensory attributes (color, aroma, taste, and overall acceptability) of laboratory produced *Iru* (Sanni & Ogbonna, 1992; Magaji *et a*l., 2025) of laboratory-produced *Iru.* Panelists were randomly selected from male and female consumers within the FCT, Abuja. The assessment was conducted by administering a Questionnaire, where the panelists rated the samples using the 9-point hedonic scale evaluation method (1=dislike extremely,9=like extremely).

Analysis of Data

Data from this study were subjected to Analysis of variance (ANOVA) to determine for significance in microbial counts during fermentation, proximate composition of laboratory produced fermented *iru* and in the sensory properties of the laboratory fermented *iru.*

RESULTS AND DISCUSSION

Results of the biochemical responses of *Bacillus* species from retail *Iru* are shown in Table 3. All isolates were Gram-positive, catalase-positive, spore-staining, motile, citrate, and Voges-Proskauer positive. Isolates were also found to be negative for sulphide, Indole test, while varied responses were observed for Methyl red, sugar fermentation, and lecithinase tests.

All isolates tested negative for blood haemolysis (gamma-hydrolysis) except for one isolate which exhibited partial hemolysis. Twelve isolates were selected as representatives of the varied responses observed biochemically for molecular characterization. Results for the molecular characterization of *Bacillus* species isolates from retail *Iru* are shown below (Figure1 and Table 2)

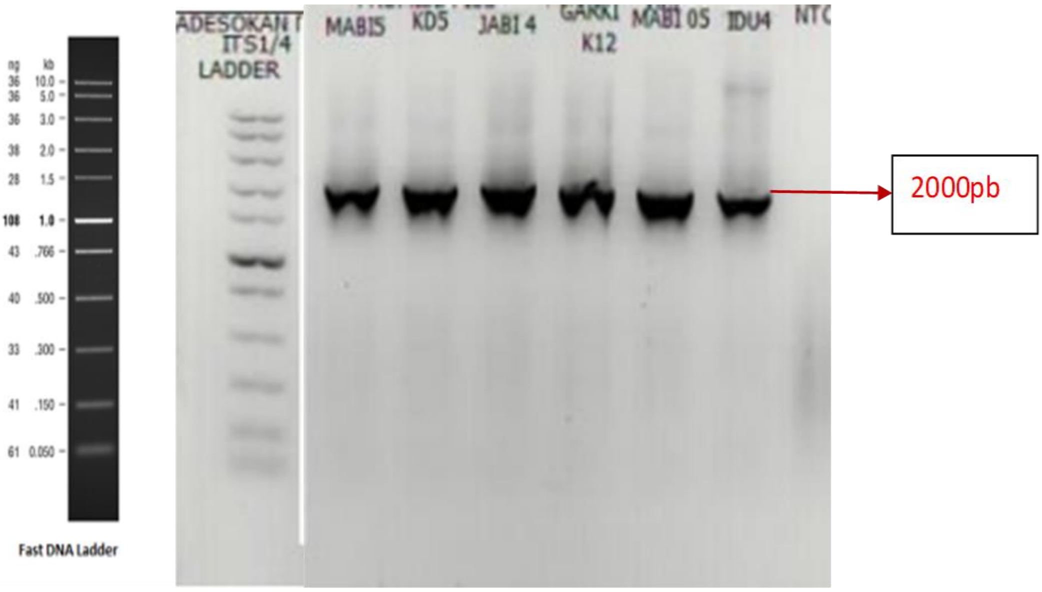

Figure 1: Amplified regions of 16S rRNA of *Bacillus* species from Retail *Iru.*Amplicon Size (2000bp). Lanes 1 (Molecular Marker of 1000bp and 10.0kb). Lanes 2-7 (Isolates of *Bacillus* species). Lane 8 (Nuclease free water a negative control).

Table 2: Molecular Identification of *Bacillus* species Isolates from Retail *Iru* based on Similarity to 16SrRNA Gene Sequences in the GenBank Database of National Centre for Biotechnological Information (NCBI) by BLAST Analysis

No	Sample ID	Confirmed Organism	% Identity	Accension No of BLAST hit	Highest Coverage (%)
1	Garki 2	*B.subtillis*	100	EU271854.1	100
2	JABI 4	*B.subtillis*	99.8	PV876475.1	99
3	KD 5	*B. licheniformis*	99.8	OL354425.1	99
4	MABI 05	*B. subtillis*	99.8	0Q600781.1	99
5	MABI 5	*B.licheniformis*	98.2	OL354425.1	98
6	IDU 4	*S.maltophilia*	99.8	CP015612.1	99
7	GAD3	*B.cytotoxicus*	99.6	CP126294.1	99

No	**Sample ID**	**Confirmed Organism**	**% Identity**	**Accension No of BLAST hit**	**Highest Coverage (%)**
8	KD 2	*B.subtilis*	99.8	CP149942.1	99
9	LOK4	*B.cytotoxicus*	99.6	CP149942.1	99
10	LOK8	*B. subtilis*	99.8	CP149942.1	99
11	WARU5	*S.maltophila*	97.0	CP149942.1	97
12	ZUBA3	*B.subtilis*	99.4	CP126294.1	99

Table 3: Biochemical Responses of *Bacillus* Species Isolates from Retail *Iru*

Test/ Samples	**KD2**	**LO4**	**AP5**	**KM5**	**LO8**	**ZU5**	**KH4**	**GA2**	**MA**	**ZU2**	**GAD3**	**KAB2**	**JAB4**	**MAB5**	**KAB3**	**KH2**	**MAB6**	**KD3**	**JA3**	**GA4**	**ZU1**
Shape	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**	**ROD**
Gram reaction	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Catalase	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Starch hydrolysis	+	+	+	M	+	+	+	+	+	M	-	-	+	M	-	M	+	+	M	M	+
Spore Staining	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	-	+	+	+	+	-	+	+	+	-	M	M	+	+	M	M	-	-	+	M	-
Hydrogen Sulphide	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Motility	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Citrate utilization	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Mannitol fermentation	-	-	+	+	+	+	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+
Fructose fermentation	-	+	+	+	+	+	-	-	-	+	+	+	+	+	-	+	-	+	+	+	+
Methyl red	-	-	-	+	+	-	-	-	-	-	+	-	-	+	IN	-	IN	-	-	+	+
Voges proskauer	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Lecithinase	-	+	IN	IN	+	+	-	+	IN	+	+	+	-	+	-	IN	+	-	IN	+	+
Identified isolate	BS	BC	BL	BL	BS	BS	BS	BS	BS	BS	BC	BC	BS	BS	BS	BS	BL	BS	BL	BL	BS

KEY:
KM = Kurudu, JA = Jabi, Lo = Lokogoma, AP = Apo, Zu = Zuba, KH = Karshi, GA = Gaduwa,
MA = Mambilla, M = moderate, IN = inconclusive, + = positive, - = negative, BS: Bacillus subtilis, BL; Bacillus licheniformis, BC: Bacillus cytotoxicus.

Microbial Counts during African Locust Beans Fermentation using *Bacillus* Species as Starter Cultures

Figure 2 shows the results of *Bacillus counts* in samples during laboratory fermentation of African locust beans using *B. subtilis* and *B. licheniformis* as single and cocultures in *Iru* production for 72h. There was a general trend of increase in counts after 24h of fermentation where highest counts were observed for sample BL with an increase of 3.1 log units from an initial level of 8.2 $\log_{10}$ cfu/g to 11.3 $\log_{10}$cfu/g. Sample BSBL recorded the lowest level, with an increase of 2.2 log units from an initial level of 8.2 $\log_{10}$ cfu/g to 10.4 $\log_{10}$ cfu/g, while sample BS had counts increased by 2.7 log units from an initial level of 8.2 $\log_{10}$ cfu/g to 10.9 $\log_{10}$ cfu/g.

Following fermentation for 48h, counts further increased for all samples, with highest increase observed for sample BSBL, which further increased by 2.8 log units to 13.2 $\log_{10}$ cfu/g. Sample BS recorded the lowest level, with an increase of 1.7 log units to 12.6 $\log_{10}$ cfu/g. Sample BL recorded an increase of 2.1 log units to 13.4 $\log_{10}$ cfu/g. Further increase in counts was observed following 72h of fermentation, where sample BS had the highest level of increase of 2.1 log units to 14.7 $\log_{10}$ cfu/g, sample BSBL increased by 1.1 log units to 14.3 $\log_{10}$ cfu/g, recording the lowest increase and sample BL recorded an increase of 1.7 log units to 15.1 $\log_{10}$ cfu/g.

pH of Samples During Fermentation of African Locust Bean Using *Bacillus* species as Starter Cultures

pH of Samples during laboratory fermentation of African locust bean using *B. subtilis* and *B. licheniformis* as single and co-cultures in iru production for 72h are shown in Figure 3. At 0hr, all samples recorded a pH value 5.1. There was a general trend of increase in pH after 24h of fermentation where highest pH value was observed for sample BS which increased from 5.1 to 7.1. Sample BL recorded the lowest level of increase, with an increase in pH by 1.3 to pH 6.4, and sample BSBL increased with a pH of 1.7 to 6.8. After fermentation for 48h, the pH further increased in all samples.

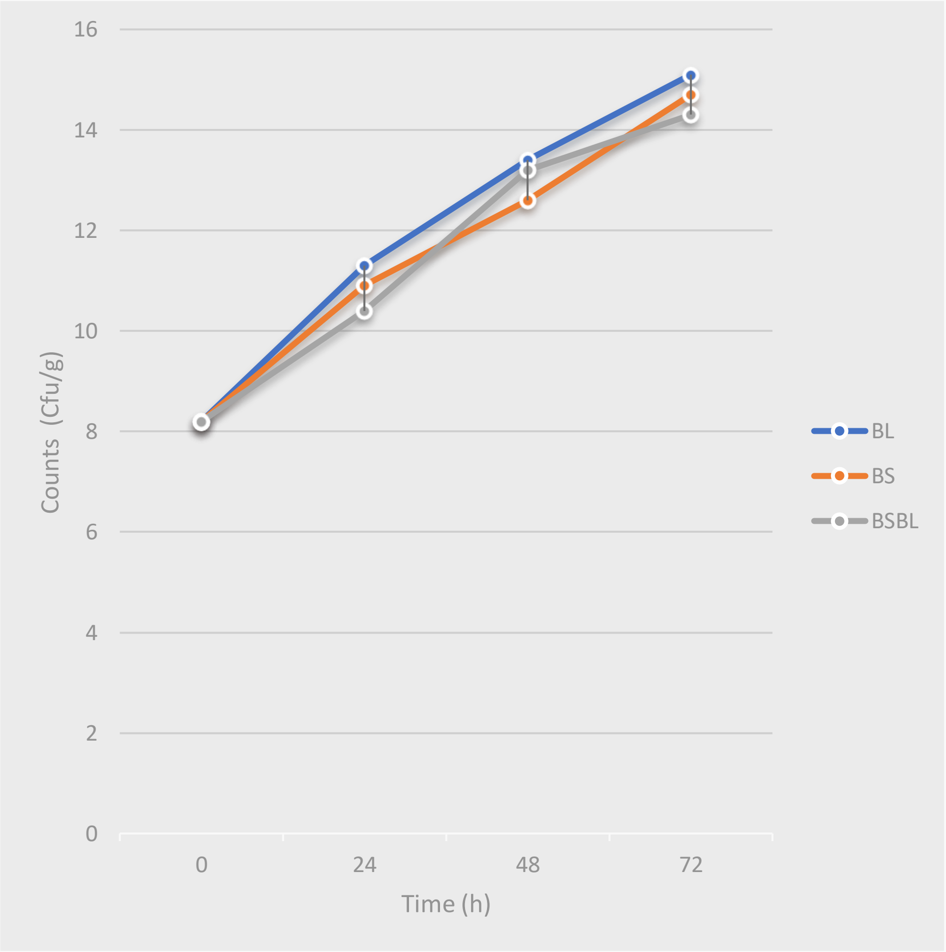

Figure 2: Microbial count during African locust bean fermentation using *Bacillus* species as starter cultures

KEY:
BS: Samples inoculated with *Bacillus subtilis* starter culture;
BL: Samples inoculated with *Bacillus licheniformis* starter culture
BSBL*:* Samples inoculated with *Bacillus subtilis* and *Bacillus licheniformis* starter culture

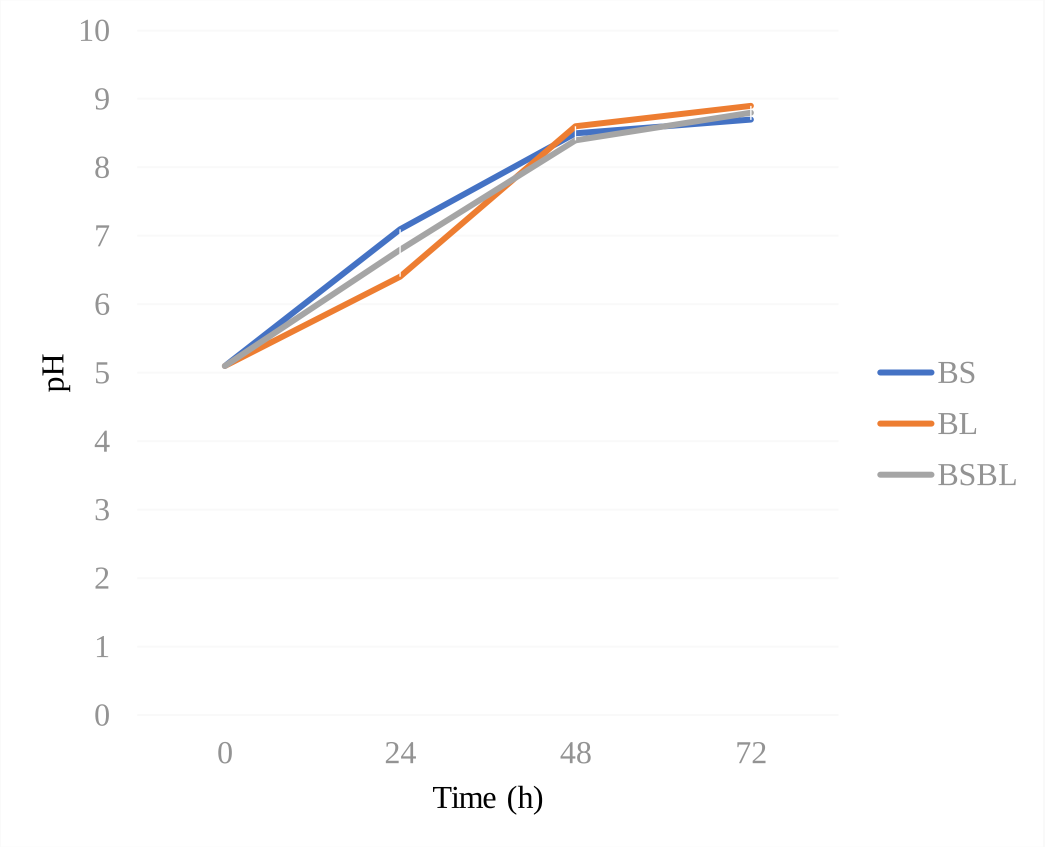

Figure 3: pH of Samples During Fermentation of African locust Beans using *Bacillus* Species as Starter culture

KEY:
BS: Samples inoculated with *Bacillus subtilis* starter culture;
BL: Samples inoculated with *Bacillus licheniformis* starter culture
BSBL*:* Samples inoculated with *Bacillus subtilis* and *Bacillus licheniformis* starter culture

Proximate Composition of Fermented African Locust Bean Using *Bacillus* Species as Starter Cultures

Means of the proximate composition of African locust bean fermented for 72h using *Bacillus subtilis*, *Bacillus licheniformis* as single and co-cultures, and unfermented African locust bean (UFB) are shown in Table 4. Variations were recorded in the compositions of ash, moisture, crude fiber, crude lipid, crude protein, and carbohydrate of samples**.** Ash content for samples ranged from 0.8% to 4.4%. Sample BS and BL recorded the highest value, while the lowest value was observed in the UFB. Moisture content ranged from 65% to 69% , where samples BS and BSBL recorded the highest values, while the lowest value was observed in the UFB. For crude fiber content, values ranged from 11.5% to 19.3%. where the UFB recorded the highest value, and the lowest value observed for sample BS. Similarly, crude protein content for samples at the end of the fermentation period ranged from 3.6% to 8.7%; whilst sample BS recorded the highest value, the lowest value was observed in UFB. Carbohydrate content for samples at the end of the fermentation period ranged from 8.7% to 14.7% with samples BL and BSBL recording the highest values, while the lowest value was observed in sample BS. Statistical analyses of data revealed statistically significant differences across the means of all samples for moisture and crude protein (P<.0.05), while variations where observed in the level of significance between means of other samples for other parameters (Table 4)

Acceptability of Fermented African Locust Bean Produced using *Bacillus* Species from Retail *Iru* as Starter Cultures

Table 5 shows the mean scores for acceptability evaluation of African locust beans fermented using *Bacillus* species from retail *Iru* as starter cultures. Mean values for aroma ranged from 7.00 to 8.00, with sample BSBL recording the highest, and the lowest value was observed in sample BL. For colour, vlaues ranged from 6.55 to 7.90 , with sample BSBL recording the highest and sample BS the lowest. Sample BSBL recorded the highest value for taste which

Table 4: Proximate Composition* of *Iru* Produced Using *Bacillus* Species From Retail *Iru* as Starter Culture

Sample	**Moisture (%)**	**Ash (%)**	**Crude Protein (%)**	**Crude Fibre (%)**	**Crude Lipid (%)**	**Carbohydrates (%)**
BL	66.68±0.1^{c}	0.95±0.0^{a}	4.11±0.0^{b}	14.65±0.1^{b}	1.70±0.0^{a}	14.72±0.1^{a}
BSBL	68.66±0.1^{b}	0.92±0.0^{b}	3.86±0.0^{c}	11.60±0.1^{c}	0.23±0.0^{c}	14.72±0.1^{a}
BS	69.34±0.1^{a}	0.95±0.0^{a}	8.75±0.0^{a}	11,45±0.1^{c}	0.80±0.0^{b}	8.68±0.1^{c}
UFB	65.10±0.1^{d}	0.84±0.0^{c}	3.60±0.0^{d}	19.33±0.1^{a}	0.30±0.0^{c}	10.81±0.1^{b}
CV%	0.2	1.2	0.8	1.1	6.0	1.2

Sample	Moisture (%)	Ash (%)	Crude Protein (%)	Crude Fibre (%)	Crude Lipid (%)	Carbohydrates (%)
LSD (5%)	0.26	0.02	0.08	0.23	0.09	0.29
F pr.	<.001	<.001	<.001	<.001	<.001	<.001
RESIDUAL d.f	6	6	6	6	6	6

KEY:
Samples within the same column with the same superscript are not significantly different (P>0.05)
*Values are means and standard deviations of triplicate samples
BS: Samples inoculated with *Bacillus subtilis* starter culture
BL: Samples inoculated with *Bacillus licheniformis* starter culture
BSBL*:* Samples inoculated with *Bacillus subtilis* and *Bacillus licheniformis* starter culture
UFB: Unfermented beans
CV: Coefficient of variation
LSD: Least significant difference
F pr: F probability
Residual d.f: Residual degree of freedom

ranged from 7.90 to 8.85 , with sample BS having the least value . Mean values for overall acceptability ranged from 7.90 to 8.90 where sample BSBL recorded the highest value, while the lowest value was observed for sample BL. Analysis of variance revealed no significant difference (P <.0.05) in the mean values for overall acceptability of samples BS and BL while the coculture sample (BSBL) was significantly different (P>0.05) from samples BS and BL

Acceptability of Fermented African Locust Bean Produced using *Bacillus* Species from Retail *Iru* as Starter Cultures

Table 5 shows the mean scores for acceptability evaluation of African locust beans fermented using *Bacillus* species from retail *Iru* as starter cultures. Mean values for aroma ranged from 7.00 to 8.00, with sample BSBL recording the highest, and the lowest value was observed in sample BL. For colour, vlaues ranged from 6.55 to 7.90 , with sample BSBL recording the highest and sample BS the lowest. Sample BSBL recorded the highest value for taste which ranged from 7.90 to 8.85 , with sample BS having the least value . Mean values for overall acceptability ranged from 7.90 to 8.90 where sample BSBL recorded the highest value, while the lowest value was observed for sample BL. Analysis of variance revealed no significant difference (P <.0.05) in the mean values for overall acceptability of samples BS and BL while the coculture sample (BSBL) was significantly different (P>0.05) from samples BS and BL

Discussion

The microbial load increased progressively throughout the 72-hour fermentation period in all samples, indicating active microbial metabolism. This confirms that *Bacillus* species play a major role in protein degradation during fermentation. Similar finding was reported by Wang *et al* . (2022), who observed increased *Bacillus* counts during *Iru* fermentation due to the dominance of *Bacillus* spp. The high microbial activity in the fermentation of sample BL compared to sample BS and sample BSBL in the first 24h of fermentation can be attributed to efficient enzyme Production, particularly proteases and lipases of *B.licheniformis* which promote substrate utilization (Wang *et al*.,2022). The surge in counts observed at 48 h with the co-culture sample could be attributed to metabolic complementation and transient co- operative growth (Akanni *et al*., 2018) whereby two co-cultured microbes support each other via exchanges of carbon, nitrogen, amino acids, and volatiles. Sample BS maintained a relatively steady increase

Table 5: Mean Values of Sensory Evaluation of Fermented African Locust Beans Produced using *Bacillus* Species from Retail *Iru* as Starter Cultures

Sample	Aroma	Color	Taste	Overall Acceptability
BS	7.10 ±0.0^b	6.55±0.1^c	7.90±0.0^b	8.05±0.0^b
BL	7.00 ± 0.0^b	7.25±0.1^b	7.95±0.0^b	7.90±0.0^b
BSBL	8.00±0.0^a	7.90±0.1^a	8.85±0.0^a	8.90±0.0^a
LSD (5%)	0.13	0.34	0.06	0.17
CV (%)	0.8%	2.1%	0.4%	0.9%
F pr.	<.001	0.001	<.001	<.001
R.d.f	4	4	4	4

KEY:
Samples within the same column with the same superscript are not significantly different (P>0.05)
*Values are means and standard deviations of triplicate samples
BS: Samples inoculated with *Bacillus subtilis* starter culture
BL: Samples inoculated with *Bacillus licheniformis* starter culture
BSBL*:* Samples inoculated with *Bacillus subtilis* and *Bacillus licheniformis* starter culture
UFB: Unfermented beans
CV: Coefficient of variation
LSD: Least significant difference
F pr: F probability
Residual d.f: Residual degree of freedom

over the fermentation period, suggesting sustained microbial activity compared to BL and BSBL.

Differences observed in the increase in pH levels across all samples over the period of fermentation suggest that each *Bacillus* species influenced the alkalinization process differently. pH values during the period of fermentation were lowest (most acidic) for sample BL, suggesting that *B. licheniformis*, as a single starter culture least supported alkaline fermentation characteristic than *B. subtilis* and the co-culture treatment (Achi, 2005) The rise in pH reflects protein hydrolysis and ammonia release from amino acid deamination, which are typical of alkaline fermentation. This agrees with the report of Achi (2005) that *Bacillus*-driven fermentations of African locust bean produce high pH values due to proteolytic activity, improving both flavor and texture.. pH values over the period of fermentation were lowest (most acidic) for sample BL at 24hrs, suggesting that *B. licheniformis* as a single starter culture least supported alkaline fermentation than *B. subtilis* single culture and their co-culture. *Bacillus licheniformis* has been documented to rapidly breakdown sugars to produce organic acids (Adebayo-Tayo *et al.*,2008), which could have accounted for the lowest pH level recorded for sample BL at 24h of fermentation.

Fermentation significantly altered the nutritional profile of the fermented African locust beanin this study. Ash, moisture, crude protein, and carbohydrate contents increased, while crude lipid and crude fiber decreased significantly. The reduction in the crude fiber could be attributed to the ability of the starter cultures to hydrolyze and metabolize them as carbon source in order to synthesize cell biomass (Famuwagun and Taiwo, 2023). The increase in ash content when compared to UFB suggests that fermentation enhanced the mineral concentration, especially for samples BS and BL which also recorded highest counts. The increased moisture content of samples at the end of the fermentation period may be attributed to exothermic nature of fermentation processes, where heat can lead to condensation (Adebayo-Tayo *et al.*, 2008). Increase in crude protein of samples in contrast to the UFB could be linked to microbial enzyme activity that breakdown protein molecules into simpler, more bioavailable forms (Felix and Francis, 2019; Zhao *et al.*, 2022). In this study, *Iru* produced from single cultures, recorded higher crude protein than *Iru* from coculture and this may be attributed to antagonistic effects arising from competition for resources leading to reduction of enzymes like proteases (Achi,2005). A report however exists which appears to associate inoculum size of *B. subtilis* and *B. licheniformis* in coculture to protein content; it was observed that soluble protein conversion rate was highest when cocultures of *B.subtilis* and *B. licheniformis* were at a ratio of 1:2 respectively (Zhao *et al.,2022*). In this study, *Bacillus subtilis* and *Bacillus licheniformis* were at a ratio of 1:1 .

Sample BSBL was liked better in terms of aroma, colour and overall acceptability. This implies that sample BSBL developed a more desirable fermentation profile, likely influenced by synergistic microbial growth, metabolic complementation and improved sensory complexity (Okolie *et al*., 2022; Bogueva &Danova, 2024). It is noteworthy however that the mean scores for all samples were above the mid-point value of 5.0 on the 9-point hedonic scale, hence it could be infered all samples in this study were generally acceptable.

CONCLUSION

Findings of this study suggests *Bacillus subtilis* and *Bacillus licheniformis* as co- cultures do not significantly impact on proximate composition of *Iru* compared to their single culture; *Bacillus subtilis* and *Bacillus licheniformis* as a coculture however yielded *Iru* of higher acceptability than those produced from their single cultures

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