AJBS Asian Journal of Biological Sciences

OPEN ACCESS

Assessment of Fermented Unripe Plantains and Soybean Blends Using Microbiological Methods

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ABSTRACT

Background and Objective: Fermentation is an old method that has been shown to enhance the nutritional makeup and ease of digestion of food. This work explores the potential of fermented unripe plantain and soybean blends as a nutrient-dense dietary source through microbiological investigations. **Materials and Methods:** Six samples were prepared using unripe plantain and soybean flours in varying ratios. Semi-solid state fermentation was conducted for 120 hrs and physicochemical parameters (pH and total titratable acidity) were assessed. Microbial identification was carried out conventionally and molecularly. Statistical analysis was performed using one-way ANOVA and Duncan's New Multiple Range Test ($p \leq 0.05$) in SPSS 22.0. **Results:** The isolated microbial strains include *Enterobacter cloacae*, *Leuconostoc mesenteroides*, *Staphylococcus aureus*, and various *Lactobacillus* and *Bacillus* species. Molecular identification was performed on *Lactobacillus plantarum* strains IMAU50267 and PT0010, and *Bacillus subtilis* strain C3a-FIIRO. Significant variations in pH, temperature, and total titratable acidity (TTA) were observed during fermentation. **Conclusion:** The results provide a viable path for the creation of novel, nutrient-dense food products by explaining the microbiological dynamics and nutritional benefits linked to fermented unripe plantain and soybean blends.

KEYWORDS

Unripe plantain (*Musa paradisiaca*), soybean (*Glycine max* (L.)), fermentation, Lactic acid bacteria, *Lactobacillus plantarum* ELISIMORE strain PQ328209, *Lactobacillus bulgaricus*, *Lactobacillus casei*, and *Leuconostoc mesenteroides*

INTRODUCTION

For millennia, fermented foods have been a mainstay in numerous cultures due to their distinct tastes, increased nutritional content, and longer shelf life[1]. The potential of fermented unripe plantains and soybean blends to provide an abundant supply of vital nutrients, such as vitamins, minerals, and proteins, has drawn the attention of many people. Fermentation improves food digestion and nutritional quality[2]. It alters the composition of intestinal microflora, inhibits the growth of pathogenic bacteria, facilitates easy digestion, boosts immunity, and increases resistance to infection[3]. However, it is crucial to guarantee the safety and value of these fermented blends, especially in light of the intricate microbial interactions that take place throughout fermentation[4]. The plantain (*Musa paradisiaca*), is a massive perennial crop that is grown in many tropical and subtropical regions of the world[5].

ISSN: 1996-3351 (Print)
https://doi.org/10.3923/ajbs.2025.541.555

Received: 28 Aug. 2024
Accepted: 21 Jan. 2025
Published: 30 Jun. 2025

Over 60 million individuals appreciate or consume plantains frequently; it gives them 25% of their daily calories from this item[6]. Unripe plantains can therefore be used as a substitute for meals high in carbohydrates, which are likely to cause consumers to develop disorders like obesity and diabetes[7]. It has been observed that diabetic patients who eat unripe plantains experienced a reduction in postprandial glucose levels[8]. Soybean (*Glycine max* (L.)) Merrill is a legume with a high protein content that lacks sulfur-containing amino acids but has high levels of lysine and tryptophan[9]. One of the most widely produced plants in the world, soybean (*Glycine max*) is primarily grown as an oil seed and pasture crop[10]. It is a member of the Fabaceae family and is recognized as a nutrient-dense food source that has steadily acquired significance in functional health foods[11]. For centuries, people have recognized the rich nutritional qualities of soybeans, which is why it has been used as food[12]. The compositions of protein, carbohydrates, oil, and ash in soybeans are higher[13]. Heat-stable soybean protein allows soy seeds to be cooked at high temperatures and fermented without changing the seeds' chemical makeup altogether[14]. Kårlund *et al.*[15] states that fermentation is a bioprocessing technique that produces desired food product quality attributes by utilizing microorganisms and their enzymes. To evaluate fermented food items, microbiological techniques are essential because they offer valuable information on the microbial communities that are present, their metabolic processes, and how these affect the product's safety and quality[16]. Understanding the microbial dynamics is crucial when it comes to fermented unripe plantain and soybean blends since it helps to optimize the fermentation process and guarantees that the finished product satisfies consumer expectations and regulatory criteria[17].

Mallappa *et al.*[18] have shown that the fermented blends' microbial diversity, composition, and functionality were determined by utilizing both contemporary molecular tools and conventional culture-based approaches. In addition, Agriopoulou *et al.*[19] stated that the presence of pathogenic bacteria and potentially spoiling microorganisms, such as probiotics, guarantee the safety and quality of finished products. Through this evaluation, this research anticipates revealing significant effects regarding how unripe plantain and soybean blends ferment and clarifies how various microorganisms affect the flavor, aroma, and nutritional value of the final product.

Furthermore, Mukherjee *et al.*[20] revealed that guidelines and strategies for the manufacture of fermented blends that satisfy safety and nutritional standards by identifying microbial markers linked to quality and safety should be established. This research unveils the guarantee of fermented unripe plantain and soybean blends as safe, nutrient-dense, and culturally relevant food products by utilizing the power of microbiological approaches. This study aimed to use microbiological techniques to thoroughly evaluate blends of fermented unripe plantains and soybeans.

MATERIALS AND METHODS

Study duration: The study was carried out from July, 2023 to July, 2024.

Compilation of samples: The study used green, matured, unripe plantains blended into 5000 g and soybean seeds blended into 2000 g. These were purchased from the Akure metropolitan area of Oja Oba, in the Nigerian State of Ondo.

Unripe plantain flour processing: The unripe plantain was cleaned by running it under sterile water and sorted for maturity. The unripe, clean plantain was then peeled, thinly sliced into pieces that were 2 mm in diameter, and let to dry in the sun for a full day. The unripe, dry plantain was subsequently put into a Model 200L090 Bentall attrition mill. After milling the flours, they were sieved into fine flours using a 0.25 mm mesh sieve and stored in an airtight container.

Soybean flour processing: Dust and pebbles were sifted out of the soybean to clean it. To extract the cotyledon from the coat, the cleaned seeds were ground coarsely. To avoid absorbing moisture, infection, and infestation by rodents or insects, they were stored in an airtight container. Before usage, the milled flour was placed in an airtight container after being sieved through a 0.25 mm mesh sieve to produce fine flour.

Plantain-soybean blend formulation: Six samples were created using the unripe plantain and soybean flours: (1000 g: 0) Sample A = unripe plantain flour (100%), (900 g: 100 g) Sample B = unripe plantain flour (90%) and soybean flour (10%), (800 g: 200 g) Sample C = unripe plantain flour (80%) and soybean flour (20%), (700 g: 300 g) Sample D = unripe plantain flour (70%) and soybean flour (30%), (600 g: 400 g) Sample E = unripe plantain flour (60%) and soybean flour (40%), (500 g: 500 g) Sample F = unripe plantain flour (50%) and soybean flour (50%).

Blends' fermentation process: Submerged liquid fermentation was used to ferment a batch of the flour blend for 120 hrs. The 1000 g of each sample was placed in cleaned, securely sealed containers, and 700 mills of sterile water were added. The fermentation was stopped by oven drying for 12 hrs at 60°C.

Analyzing the samples microbiologically: Nutrient agar (NA) was used to measure the total number of bacteria present, and De Man-Rogosa-Sharpe agar (MRSA) was employed to separate lactic acid bacteria. Utilizing the proper pour plate and serial dilution methods, microorganisms were counted. Anaerobic incubation of the De Man-Rogosa-Sharpe agar plates was conducted for 18 to 48 hrs at 32°C, while the bacterial culture was cultured at 37°C for 18 to 24 hrs. The biochemical and morphological observations were used to characterize the organisms using the techniques described by Tindall *et al.*[21] and Cheesbrough[22].

Identification of isolates molecularly: Identification was carried out following the methodology outlined by Tindall *et al.*[21] and Liaqat and Eltem[23].

Temperature, pH and TTA determination: Using a pocket-sized pH meter, the pH of each fermenting sample was measured every 24 hrs. The pH meter and the thermometer were manufactured by BIC Farms Abeokuta, Ogun State, Nigeria. In 10 mL of distilled water, 1 g of the sample was dissolved and then filtered. The pH meter's electrode was dipped into the sample solution after it had been calibrated using buffer solutions with pH values of 4, 7 and 9. The measured pH was then recorded in triplicate. At 24 hrs intervals, the fermenting samples' total titratable acidity was measured. A beaker was filled with 2 g of the ground sample. It was combined with 20 mL of distilled water, stirred and filtered. Two drops of phenolphthalein indicator were added to a beaker containing 10 mL of the filtrate, after which it was measured. After titrating this with a 0.1 M solution of sodium hydroxide (NaOH), the titer value was determined. Lactic acid percentage (%) was used to express total titratable acidity.

The formula used to determine acidity was[24]:

$$\text{TTA} = \frac{\text{Titer value}}{9\ \text{mg}} \times 100$$

The samples' pH and TTA were measured using the procedure outlined by AOAC in 2012. A mercury-filled bulb thermometer was used to measure the temperature. Every 24 hrs, the thermometer was put into the substrate to check the temperature.

Data analysis using statistics: Triplicate results of every analysis were carried out. One-way Analysis of Variance (ANOVA) was performed on the collected data while Duncan's New Multiple Range Test (DMRT) was used to find mean differences at the significance level of $p \leq 0.05$. All analyses of the data were conducted using SPSS 22.0.

RESULTS

pH variations during the fermentation of blends of unripe plantains and soybean flour: The pH of the fermented blends showed noticeable variations, which are shown in Fig. 1. With an increase in fermentation time, the pH of the fermented blends decreased.

Variations in total titratable acidity during unripe plantain and soybean flour blend fermentation: Figure 2 shows the variations in unripe plantain and soybean blends' total titratable acidity (TTA) throughout fermentation. It was found that the samples' total titratable acidity (TTA) increased along with the blends' fermentation hours.

Variations in temperature during the fermentation of soybean flour blends and unripe plantains: Figure 3 illustrates the temperature variations that occur as unripe plantains and soybeans ferment. The temperature of the samples slightly increased with an increase in fermentation time. Sample A had an initial temperature of 28±0.00°C, this increased to 30±0.00°C at 120 hrs. The temperature of sample B increased from 29±0.00°C at 0 hr to 30±0.01°C at 120 hrs. Sample C had an initial temperature of 27±0.01°C at 0 hr, followed by an increase of 31±0.00°C at 120 hrs. The initial temperature of sample D was 28±0.00°C at 0 hr, this increased to 32±0.00°C at 120 hrs. The temperature of sample E at 0 hr was 30±0.00°C, it increased to 32±0.00°C at 120 hrs. The temperature of sample F at 0 hr was 29±0.00°C, it increased to 32±0.00°C at 120 hrs.

Changes in bacterial load during fermentation of unripe plantain and soybean blends: The change in bacterial load during the fermentation of unripe plantain and soybean blends is presented in Table 1. Sample A had an initial bacterial load of 1.53×10^5 CFU/g at 0 hr. This increased to 3.86×10^5, 2.67×10^5 and 5.36×10^5 CFU/g at 24, 48 and 72 hrs, respectively. This was followed by a subsequent decrease in bacterial population to 2.57×10^5 and 2.61×10^5 CFU/g at 96 and 120 hrs. The bacterial count of samples B, C, E and

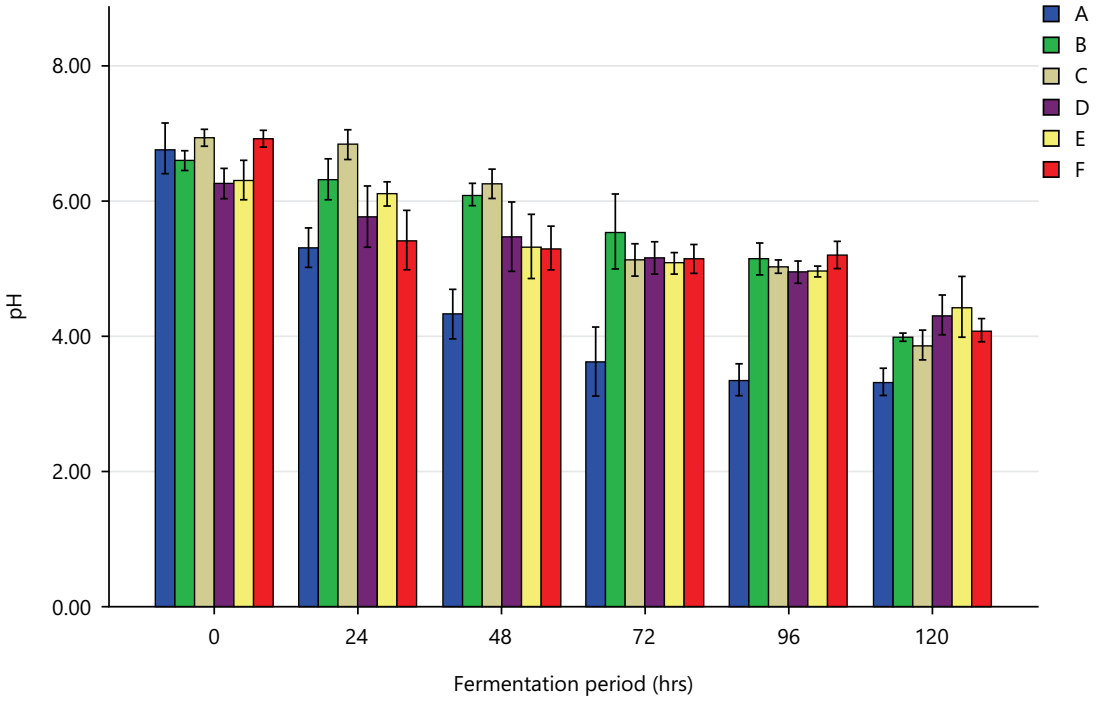

Fig. 1: pH variations during the fermentation of blends of unripe plantains and soybean flour
A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%), F: Unripe plantain (50%): Soybean (50%) and Error bars: 65% CI

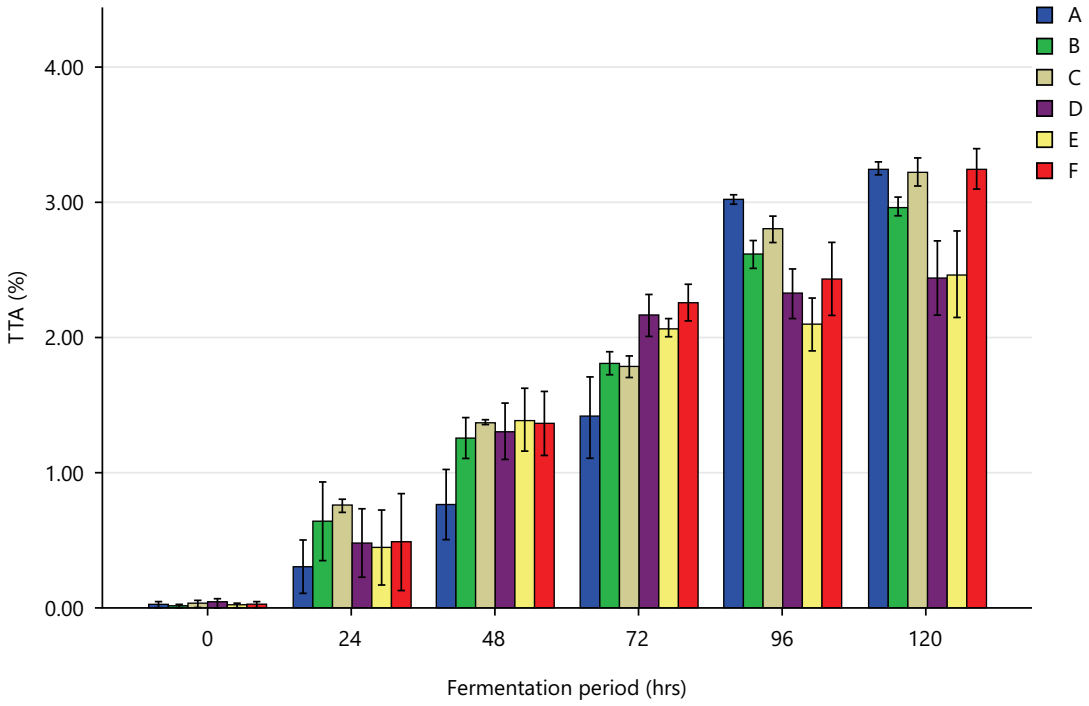

Fig. 2: Variations in total titratable acidity (TTA) during unripe plantain and soybean flour blend fermentation

A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%), F: Unripe plantain (50%): Soybean (50%) and Error bars: 50% CI

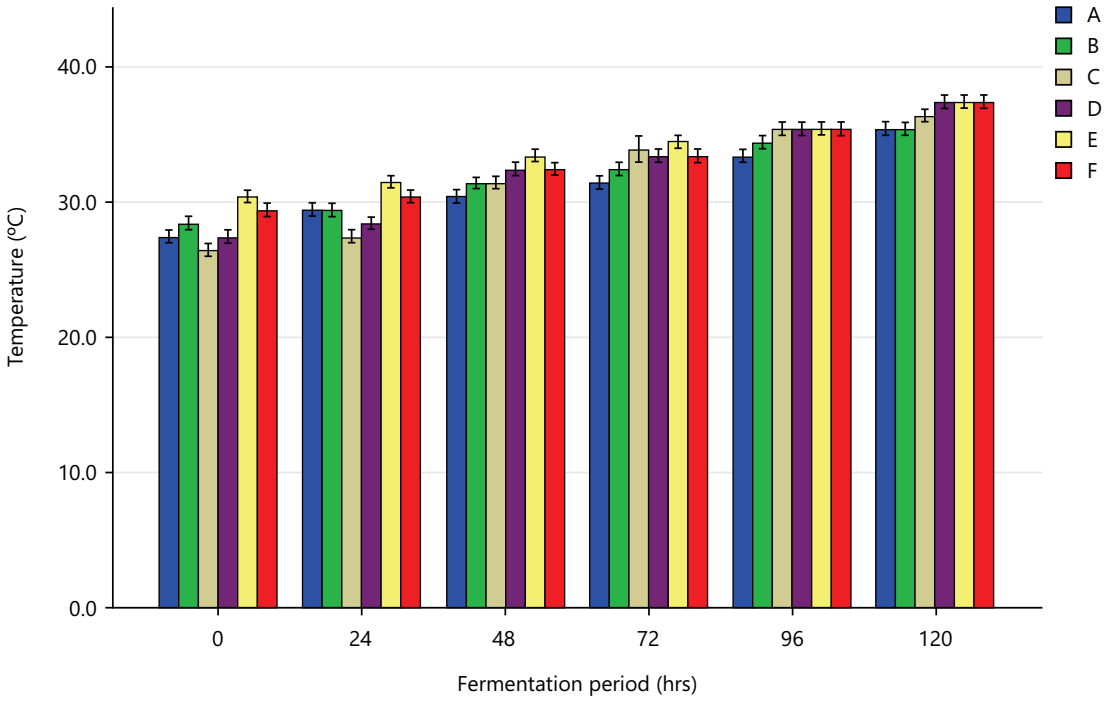

Fig. 3: Variations in temperature during the fermentation of soybean flour blends and unripe plantains

A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%) and F: Unripe plantain (50%): Soybean (50%) and Error bars: 50% CI

Table 1: Changes in the bacterial load (CFU/g) during the fermentation of blends of unripe plantains and soybeans (10^5)

	Fermentation time (hrs)					
Sample	0	24	48	72	96	120
A	1.53±0.19^{ab}	3.86±0.09^{bc}	2.67±0.10^{b}	5.36±0.51^{ab}	2.57±0.52^{a}	2.61±0.49^{a}
B	2.06±0.36^{bc}	2.27±0.39^{a}	3.48±0.39^{c}	4.77±0.25^{a}	2.67±0.09^{a}	2.37±0.53^{a}
C	2.23±0.04^{c}	4.46±0.37^{c}	3.56±0.23^{c}	5.39±0.01^{ab}	2.68±0.39^{a}	2.15±0.63^{a}
D	1.26±0.37^{a}	3.47±0.09^{b}	2.65±0.07^{b}	5.58±0.30^{b}	3.33±0.45^{a}	2.65±0.35^{a}
E	1.86±0.08^{abc}	3.62±0.03^{b}	3.04±0.06^{bc}	5.29±0.13^{ab}	2.79±0.41^{a}	2.46±0.37^{a}
F	1.55±0.21^{ab}	3.37±0.53^{b}	2.01±0.30^{a}	5.89±0.11^{b}	2.37±0.79^{a}	2.07±0.81^{a}

Values represent three determinations' Mean±Standard Deviation, Significant differences ($p<0.05$) were observed in values that are not indicated in the same column by the same superscripts, A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%) and F: Unripe plantain (50%): Soybean (50%)

Table 2: Variations in the lactic acid bacterial load during the fermentation of blends of unripe plantains and soybeans (10^5)

	Fermentation time (hrs)					
Sample	0	24	48	72	96	120
A	0.00±0.00	0.00±0.00	1.55±0.21^{a}	2.50±0.28^{a}	2.70±0.28^{a}	2.35±0.50^{a}
B	0.00±0.00	0.00±0.00	1.60±0.13^{a}	2.40±0.43^{a}	2.51±0.28^{a}	2.18±0.12^{a}
C	0.00±0.00	0.00±0.00	1.79±0.41^{a}	2.65±0.36^{a}	2.65±0.36^{a}	2.25±0.07^{a}
D	0.00±0.00	0.00±0.00	1.75±0.35^{a}	2.35±0.07^{a}	2.81±0.23^{a}	2.81±0.57^{a}
E	0.00±0.00	0.00±0.00	1.50±0.28^{a}	2.71±0.16^{a}	2.70±0.28^{a}	2.50±0.28^{a}
F	0.00±0.00	0.00±0.00	1.75±0.21^{a}	2.74±0.34^{a}	3.19±0.55^{a}	2.39±0.68^{a}

Values represent three determinations' Mean±Standard Deviation, Significant differences ($p<0.05$) are observed in values that are not indicated in the same column by the same superscripts, A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%) and F: Unripe plantain (50%): Soybean (50%)

Falso increased from 0 to 72 hrs followed by a decrease between 96 and 120 hrs. The bacterial load of sample D increased from 1.26×10^5 to 5.58×10^5 CFU/g from 0 to 72 hrs while it decreased to 3.33×10^5 and 2.65×10^5 CFU/g at 96 and 120 hrs, respectively.

Changes in lactic acid bacteria load during fermentation of unripe plantain and soybean blends: Table 2 shows the changes in the lactic acid bacteria population during the fermentation of unripe plantain and soybean blends. No lactic acid bacteria growth was recorded at 0 and 24 hrs for all the samples. The lactic acid bacterial load of sample A increased from 1.55×10^5 CFU/g at 48 hrs to 2.70×10^5 CFU/g at 96 hrs, but at 120 hrs there was a decline in the load. The same trend was observed in samples B to F.

Identified bacterial species: During the fermentation of unripe plantain and soybean blends, the following lactic acid bacteria have been conventionally isolated and identified, *Lactobacillus plantarum, Lactobacillus bulgaricus, Streptococcus lactis, Leuconostoc mesenteroides, Lactobacillus fermentum* and *Lactobacillus casei*. Four gram-positive bacteria were isolated and identified these include; *Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis* and *Bacillus cereus*. One strain of Gram-negative bacteria was isolated and identified. This includes *Enterobacter cloacae*. These are shown in Table 3. The molecular identity of the common bacteria that were isolated and identified is displayed in Table 4. Two different strains of *Lactobacillus plantarum* were molecularly identified. They include; *Lactobacillus plantarum* ELISIMORE strain PQ328209 and *Lactobacillus plantarum* SIMIORE strain PQ326135. One strain of Gram-negative bacteria was molecularly identified-*Bacillus subtilis* ESO strain PQ326136. This table shows the strain number of each isolate with their percentage identity and ascension number.

Figure 4 illustrates the bacterial gene amplification that occurs during the fermentation of blends of unripe plantains and soybeans. Gel electrophoresis displays the amplification of the 16S rRNA gene, with a product size of approximately 1500 bp.

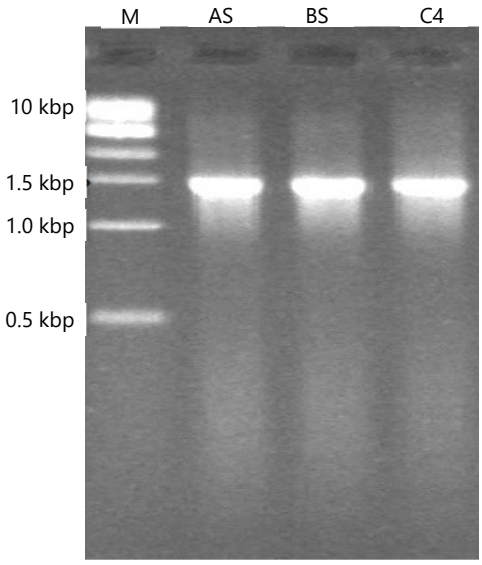

Fig. 4: 16S rRNA gene amplification image from gel electrophoresis of bacteria presents during unripe plantain fermentation: Soybean blends

Gel image showing amplification of the 16S rRNA gene at about 1500 bp and Lane M is a 1 kbp DNA ladder

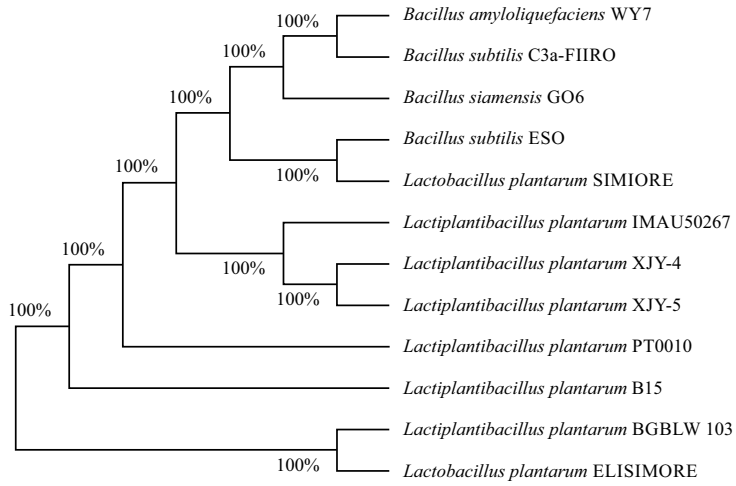

Fig. 5: Phylogenetic tree illustrating the relationships between the isolates

Table 3: Bacterial biochemical characteristics identified during unripe plantain and soybean blend fermentation

Isolates	Gram staining	Shape	Catalase	Citrate	Coagulase	Motility	Indole test	Gas production	Glucose	Lactose	Sucrose	Arbinose	Probale bacteria
1	-	Rod	+	+	-	+	-	+	+	+	+	+	*Enterobacter cloacae*
2	+	Rod	-	+	-	-	-	-	+	+	-	-	*Leuconostoc mesenteroides*
3	+	Cocci	+	-	-	-	-	-	+	-	-	-	*Micrococcus luteus*
4	+	Cocci	+	+	+	-	-	-	+	+	+	-	*Staphylococcus aureus*
5	+	Cocci	-	+	+	-	-	+	+	+	+	+	*Streptococcus lactis*
6	+	Rod	-	+	+	-	-	+	+	+	+	+	*Lactobacillus fermentum*
7	+	Rod	+	+	-	+	-	+	+	-	-	+	*Bacillus subtilis*
8	+	Rod	+	+	-	+	-	+	+	+	+	+	*Bacillus cereus*
9	+	Rod	-	+	-	-	-	-	+	+	+	+	*Lactobacillus plantarum*
10	+	Rod	-	-	-	-	-	-	+	+	-	+	*Lactobacillus bulgaricus*
11	+	Rod	-	+	-	-	-	+	+	+	+	+	*Lactobacillus casei*

+: Positive reaction and -: Negative reaction

Table 4: Molecular identity of the bacteria linked to the fermentation of mixes of unripe plantains and soybeans

Bacterial isolate	Identity (%)	Ascension number
Lactobacillus plantarum ELISIMORE strain PQ328209	1000	MF623272.1
Lactobacillus plantarum SIMIORE strain PQ326135	100	KX074211.1
Bacillus subtilis ESO strain PQ326136	100	MW577298.1

The unidentified isolates are shown in the gel electrophoresis image in Fig. 4. The phylogenetic tree that illustrates the relationships between the isolates is shown in Fig. 5. The phylogenetic tree indicates that the strain of *Bacillus subtilis* ESO is related to *Bacillus amyloliquefaciens*. The relatedness of *Lactobacillus plantarum* ELISIMORE strain PQ328209 and *Lactobacillus plantarum* SIMIORE strain PQ326135 is shown in the phylogenetic tree in Fig. 5.

Bacterial succession during fermentation of unripe plantain and soybean blends: The succession of bacteria during the fermentation of cassava and African yam bean blends is shown in Table 5. In sample A, *Bacillus subtilis* was isolated at 0 and 24 hrs, however, it was no longer present at 48 hrs. *Lactobacillus plantarum* was identified at 48, 72 and 96 hrs, while *Streptococcus lactis* and *Lactobacillus casei* were observed at 120 hrs. In sample B, *Enterobacter cloacae* and *Staphylococcus aureus* were isolated at 0 and 24 hrs. *Leuconostoc mesenteroides*, *Bacillus subtilis*, *Lactobacillus casei* and *Bacillus cereus* were observed at 48 and 72 hrs. *Streptococcus lactis*, *Bacillus cereus*, *Lactobacillus casei* and *Streptococcus lactis* were isolated between 96 and 120 hrs. In sample C, *Staphylococcus aureus* and *Bacillus cereus* were found at 0 hr. *Bacillus cereus*, *Micrococcus luteus* and *Staphylococcus aureus* were found at 0 and 24 hrs. *Bacillus cereus* and *Lactobacillus fermentum* were isolated at 48 and 72 hrs. *Lactobacillus fermentum*, *Leuconostoc mesenteroides* and *Bacillus cereus* were isolated at 96 and 120 hrs. In sample D, *Micrococcus luteus* and *Bacillus cereus* were isolated at 0 hr. *Bacillus subtilis*, *Bacillus cereus* and *Streptococcus lactis* were isolated within 24 to 96 hrs. But at 120 hrs only *Streptococcus lactis* was isolated. In sample E, *Staphylococcus aureus*, *Bacillus cereus* and *Bacillus subtilis* were isolated from 0 to 24 hrs. *Bacillus cereus*, *Bacillus subtilis* and *Lactobacillus plantarum* were isolated from 48 to 96 hrs.

Lactobacillus plantarum and *Lactobacillus casei* were the only isolates observed at 120 hrs. In sample F, *Bacillus subtilis*, *Staphylococcus aureus* and *Micrococcus luteus* were isolated between 0 and 48 hrs. At 72, 96 and 120 hrs, *Bacillus subtilis*, *Leuconostoc mesenteroides* and *Lactobacillus bulgaricus* were isolated.

Changes in fungal load during fermentation of unripe plantain

Soybean blends: The changes in fungal count are represented in Table 6. Sample A to sample F had no fungal population at 0 hr. At 24 hrs there was an initial increase in the fungal growth of sample A (1.43×0^5 CFU/g) which increased to 6.12×10^5 CFU/g at 24 hrs. This was followed by a decrease in population to 3.7×104 CFU/g at 48 hrs and a subsequent decrease to 4.22×10^5, 3.99×10^5 and 1.26×10^5 CFU/g at 72, 96 and 120 hrs, respectively. The initial fungal load of sample B was 1.32×10^5 CFU/g at 24 hrs. This increased to 5.97×10^5 and 5.85×10^5 CFU/g at 48 and 72 hrs. This was followed by a decrease to 4.55×10^5 and 0.91×10^5 CFU/g at 96 and 120 hrs, respectively. Sample C had initial population of 1.13×10^5 CFU/g at 24 hrs which increased to 5.86×10^5 and 4.81×10^5 CFU/g at 48 and 72 hrs. This was followed by decrease to 4.02×10^5 and 1.03×10^5 CFU/g at 96 and 120 hrs, respectively. The initial fungal count of sample D was 1.76×10^5 CFU/g at 24 hrs, it increased to 6.12×10^5 CFU/g at 48 hrs and decreased to 5.75×10^5, $5.05\text{x}10^5$ and 1.37×10^5 CFU/g at 72, 96 and 120 hrs, respectively. Sample E had initial load of 1.13×10^5 CFU/g at 24 hrs. It increased to 5.51×10^5 CFU/g at 48 hrs and subsequently decreased to 4.8910^5, 4.35×10^5 and 0.99×10^5 CFU/g at 72, 96 and 120 hrs, respectively. Sample F recorded initial fungal load of 1.60×10^5 CFU/g at 24 hrs. This increased to 6.38×10^5 CFU/g at 48 hrs this was followed by a decrease in population to 5.17×10^5 CFU/g at 72 hrs and 4.85×10^5 and 1.13×10^5 CFU/g at 96 and 120 hrs, respectively.

Table 5: Bacterial succession in the fermentation of blends of unripe plantains and soybeans

	Fermentation time (hrs)					
Sample	0	24	48	72	96	120
A	*Bacillus subtilis*	*Bacillus subtilis*	*Lactobacillus plantarum*, *Bacillus subtilis*	*Lactobacillus plantarum*, *Bacillus subtilis*	*Lactobacillus plantarum*, *Bacillus subtilis*	*Streptococcus lactis*, *Lactobacillus casei*
B	*Enterobacter cloacae*	*Staphylococcus aureus*, *Enterobacter cloacae*	*Leuconostoc mesenteroides*, *Bacillus subtilis*	*Lactobacillus casei*, *Bacillus cereus*	*Streptococcus lactis*, *Bacillus cereus*	*Lactobacillus casei*, *Streptococcus lactis*
C	*Staphylococcus aureus*, *Bacillus cereus*	*Bacillus cereus*, *Micrococcus luteus*, *Staphylococcus aureus*	*Bacillus cereus*, *Lactobacillus fermentum*	*Bacillus cereus*, *Lactobacillus fermentum*	*Lactobacillus fermentum*, *Leuconostoc mesenteroides*	*Bacillus cereus*, *Lactobacillus fermentum*
D	*Micrococcus luteus*, *Bacillus cereus*	*Bacillus subtilis*, *Bacillus cereus*	*Bacillus cereus*, *Streptococcus lactis*	*Bacillus cereus*, *Streptococcus lactis*	*Streptococcus lactis*, *Bacillus cereus*	*Streptococcus lactis*
E	*Staphylococcus aureus*, *Bacillus cereus*	*Staphylococcus aureus*, *Bacillus cereus*	*Bacillus cereus*, *Lactobacillus plantarum*	*Bacillus subtilis*, *Lactobacillus plantarum*,	*Lactobacillus plantarum*, *Bacillus subtilis*	*Lactobacillus plantarum*, *Lactobacillus casei*
F	*Bacillus subtilis*	*Staphylococcus aureus*, *Micrococcus luteus*, *Bacillus subtilis*	*Bacillus subtilis*	*Bacillus subtilis*, *Leuconostoc mesenteroides*	*Lactobacillus bulgaricus*, *Leuconostoc mesenteroides*	*Leuconostoc mesenteroides*, *Bacillus subtilis*, *Lactobacillus bulgaricus*

A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%) and F: Unripe plantain (50%): Soybean (50%)

Table 6: Variations in fungal load (CFU/g) during unripe plantain-soybean blend fermentation (10^5)

	Fermentation time (hrs)					
Sample	0	24	48	72	96	120
A	0.00±0.00	1.43±0.18^{a}	6.13±0.05^{a}	4.22±0.17^{a}	3.99±0.42^{a}	1.26±0.08^{ab}
B	0.00±0.00	1.32±0.17^{a}	5.97±0.09^{a}	5.85±0.21^{c}	4.55±0.49^{ab}	0.91±0.29^{a}
C	0.00±0.00	1.13±0.05^{a}	5.86±0.23^{a}	4.81±0.30^{b}	4.02±0.02^{a}	1.03±0.04^{ab}
D	0.00±0.00	1.76±0.37^{a}	6.12±0.03^{a}	5.75±0.21^{c}	5.05±0.07^{b}	1.37±0.09^{b}
E	0.00±0.00	1.13±0.04^{a}	5.51±0.28^{a}	4.89±0.26^{b}	4.35±0.07^{ab}	0.99±0.13^{a}
F	0.00±0.00	1.60±0.42^{a}	6.38±0.83^{a}	5.17±0.09^{b}	4.85±0.070^{b}	1.13±0.04^{ab}

Values represent three determinations' Mean±Standard Deviation, Significant differences ($p<0.05$) are observed in values that are not indicated in the same column by the same superscripts, A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%) and F: Unripe plantain (50%): Soybean (50%)

Table 7: Morphology and microscopy of the fungi identified during the fermentation of unripe plantains and soybeans

Morphology of colonies on agar	Microscopic structure	Suspected fungi
Typically, the colonies have smooth walls and a blue-green color	Conidial heads that are uniseriate and columnar, with phialides restricted to the top two-thirds of the vesicle and curving roughly parallel to one another	*Aspergillus fumigatus*
Aerial mycelia of the colonies were initially white, but they promptly expanded and became fluffy and velvety Conidia heads of the colonies often developed from dark brown to black, without any reversal of color	Conidia heads are big, globose and spherical. As they mature, they divide into loose columns of conidia chains. Conidiophores that emerge from the substratum are primarily colorless. Crushed into particles resembling smooth, brown cane. Metulae are typically present on phialides that are carried directly on globose vesicles	*Aspergillus niger*
Whitish, creamy and soft	Septate, mostly submerged hyphae are seen to be creeping	*Geotricum candidum*
Colonies are smooth, spherical and have a cream color	Pseudo hyphae are present	*Candida utilis*
Foul-smelling and dark pinkish color	Cream-colored, smooth and umbonal with a complete edge. Dividing cells that do not produce pseudo mycelium but instead produce ascospores	*Saccharomyces cerevisiae*

Table 8: Fungal succession in the fermentation of blends of unripe plantains and soybeans

	Fermentation time (hrs)					
Sample	0	24	48	72	96	120
A	*Aspergillus niger*	*Aspergillus niger*	*Candida utilis*	*Candida utilis*	*Candida utilis*, *Saccharomyces cerevisiae*	*Candida utilis*
B	*Aspergillus niger*	*Aspergillus niger*	*Candida utilis*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*, *Candida utilis*
C	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Geotricum candidum*	*Geotricum candidum*	*Geotricum candidum*, *Saccharomyces cerevisiae*	*Geotricum candidum*, *Saccharomyces cerevisiae*
D	*Geotricum candidum*	*Geotricum candidum*	*Geotricum candidum*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
E	*Aspergillus fumigatus*	*Aspergillus fumigatus*	*Aspergillus fumigates*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*	*Saccharomyces cerevisiae*
F	*Geotricum candidum*	*Geotricum candidum*	*Geotricum candidum*,	*Geotricum candidum*	*Saccharomyces cerevisiae* *Geotricum candidum*,	*Geotricum candidum*, *Saccharomyces cerevisiae*

A: Unripe plantain (100%), B: Unripe plantain (90%): Soybean (10%), C: Unripe plantain (80%): Soybean (20%), D: Unripe plantain (70%): Soybean (30%), E: Unripe plantain (60%): Soybean (40%) and F: Unripe plantain (50%): Soybean (50%)

Morphological and microscopic characteristics of molds and yeast isolated during fermentation of unripe plantain and soybean blends: Table 7 shows the morphological and microscopic characteristics of molds and yeast that were isolated during the fermentation of unripe plantain and soybean blends. The molds that were identified include: *Aspergillus fumigatus*, *Aspergillus niger* and *Geotricum candidum*. *Candida utilis* and *Saccharomyces cerevisiae* were the two yeasts identified during the fermentation of the blends.

Fungal succession during fermentation of unripe plantain and soybean blends: The succession of fungi during the fermentation of unripe plantain and soybean blends is shown in Table 8. In sample A, *Aspergillus niger* was isolated at 0 and 24 hrs. *Candida utilis* was isolated at 48, 72, 96 and 120 hrs. At 96 hrs *Candida utilis* and *Saccharomyces cerevisiae* were found in sample A. In sample B, *Aspergillus niger* was present at 0 and 24 hrs. *Candida utilis* and *Saccharomyces* were isolated at 48, 72, 96 and 120 hrs. In sample C, *Aspergillus fumigatus* and *Geotrichum candidum* were isolated at 0, 24, 48 and 72 hrs, while *Geotrichum candidum* and *Saccharomyces cerevisiae* were isolated between 96 and 120 hrs. In sample D, *Geotrichum candidum* was present between 0 and 48 hrs while *Saccharomyces cerevisiae* was only isolated between 72 and 120 hrs. In sample E, *Aspergillus fumigatus* was isolated between 0 and 48 hrs. *Saccharomyces cerevisiae* was present between 72 and 120 hrs. In sample F, *Geotrichum candidum* was present between 0 to 72 hrs. *Geotrichum candidum* and *Saccharomyces cerevisiae* were present at 96 and 120 hrs.

DISCUSSION

It was observed that there was a reduction in the pH of fermented unripe plantain and soybean blends and also an increase in the TTA of the blends was also noticed. The fermentation of unripe plantain and soybean blends may have reduced the pH and increased TTA as a result of the formation of organic acids by microorganisms[24]. Comparable results were noted by Tufa *et al.*[25], who observed that when prepared complementary foods fermented, there was a decrease in pH and a rise in TTA. The rise in temperature that occurs during the samples' fermentation is a sign that energy is being released[26]. This energy is the consequence of active microbial activities brought on by enhanced microbial biomass, which leads to the comparatively abundant availability of nutrients in primary metabolism[27]. The temperature of the fermented samples increased between 0 to 120 hrs. Similar results were seen by Gadaga *et al.*[28], who recorded that traditional meals ferment at a higher temperature. When unripe plantain and soybean blends were fermented for 120 hrs, a wide variety of microorganisms were identified. When compared to cereals that were fermented alone, products enriched with legumes exhibited higher microbial populations and variety, as shown by the study reported by Garrido-Galand *et al.*[29]. Bacteria and fungi, which were present in the fermenting environment, have been linked to the fermentation of several protein and carbohydrate-based meals in Nigeria[30].

For ages, fermented foods have been an essential part of human diets, providing not just distinct tastes but also health benefits[31]. Traditional fermented foods have been increasingly popular recently due to their possible health benefits, which include improved digestive health and nutrient bioavailability[32]. This discourse explores into the microbiological evaluation of the blends: fermented unripe plantains and soybeans. In addition to being a nutrient-dense dietary choice, these blends have potential as a tool for investigating the microbial dynamics of fermentation.

To evaluate fermented foods' safety and possible health benefits, it is essential to comprehend their microbial makeup[33]. Many microorganisms are involved in the fermentation process when it comes to fermented unripe plantains and soybean blends[34]. Lactic acid bacteria (LAB) are important because they ferment carbohydrates, which increases the final product's acidity[35]. These blends' safety is enhanced by the acidic environment's ability to prevent the growth of harmful microorganisms[36]. Moreover, yeast species could also exist, taking part in the fermentation process and impacting the product's flavor quality[37].

A number of techniques are used in the microbiological evaluation of fermented foods to identify the microbial makeup and guarantee product safety[38]. Two culture-based techniques for counting viable microorganisms, like yeast and LAB, are plate counts and most probable number (MPN) testing[38]. These techniques yield important details regarding the number of healthy bacteria and possible toxins in the final product[39]. Molecular methods, such as next-generation sequencing (NGS) and polymerase chain reaction (PCR), provide a more thorough understanding of the variety of microbes found in fermented blends[40]. These techniques provide a thorough insight into the microbial ecology within the product by identifying both culturable and non-culturable bacteria by focusing on particular genetic markers or sequencing the complete microbial population[41].

Although the process of fermentation can increase food product safety by making it more difficult for pathogenic entities to thrive, it is crucial to make sure that fermented blends adhere to microbiological safety guidelines[20]. Fermented foods are evaluated for safety using microbiological criteria, which include limitations for particular bacteria populations including *Salmonella* species*, Escherichia coli* and total coliforms[42,43]. To ensure product quality and prevent the growth of unsafe bacteria, these factors must be regularly monitored during production and storage.

CONCLUSION

Conclusively, this research reveals that understanding the microbial makeup of fermented unripe plantain and soybean blends, guaranteeing product safety and streamlining fermentation operations all depend on the microbiological evaluation of these blends. Researchers can make significant progress toward the production of safe and nutrient-dense fermented foods by utilizing a combination of molecular and culture-based approaches to get useful insights into the microbial dynamics of these blends. Moreover, further investigation into this area may reveal new microbial strains with probiotic potential, which would increase the health advantages of fermented foods.

SIGNIFICANCE STATEMENT

This study on the microbiological analysis of fermented unripe plantains and soybean blends is significant for ensuring the safety, quality and nutritional benefits of these products. The findings will provide valuable insights into the role of microbial activity in fermentation, with implications for public health, nutrition and food science. The research holds promising potential for the development of functional foods, commercialization opportunities and the scaling up of production to make fermented plantain soybean blends a viable food product, particularly in regions with high malnutrition or a strong reliance on plant-based diets. Additionally, these blends could serve as a foundation for children's complementary weaning foods or fortified diets.

ACKNOWLEDGMENTS

We appreciate the efforts of the staff and technologists in the Biological Sciences Department at Augustine University, Ilara-Epe, Lagos State, Nigeria and Microbiology Department, Federal University of Technology Akure, Ondo State, Nigeria.

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