UJMR, Conference Special Issue Vol. 10 No. 3. June, 2025, pp. 185 - 197

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

https://doi.org/10.47430/ujmr.25103.020

Received: 09 April 2025 **Accepted:** 17 June 2025

Assessment of Microbial and Nutritional compositions of Fermented Plantain flour across the three Senatorial districts in Ogun State, Nigeria

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Abstract

Flour is a carbohydrate-rich fiber source that is exposed to microbial contamination and nutritional alterations during preparation. This study investigates samples collected from six markets across Ogun State, Nigeria, covering the three senatorial districts (Ogun Central, East, and West). Serial dilution was employed for the total bacteria count, while identification was carried out using 16S rRNA sequencing. Nutritional assessments of the flour samples were also evaluated using techniques to determine the crude protein, fat, fiber, ash content, and moisture content of the flour samples. The highest TBC was recorded in samples from Waterside (3.5 × 10^4 CFU/ml), while the lowest was found in Ilaro samples (1.8 × 10^4 CFU/ml) with a mean total bacterial count of 2.73 × 10^4 CFU/ml. Molecular identification revealed *Bacillus licheniformis*, *Providencia rettgeri*, *Pusillimonas spp.*, *Bacillus thuringiensis*, *Bacillus tropicus*, *Bacillus pseudomycoides*, and *Lysinibacillus spp*. Most of the isolates showed resistance to the antibiotics employed in the study, while *Bacillus thuringiensis* and *Lysinibacillus spp*. were susceptible to Ofloxacin. Nutritional analysis of the flour samples revealed high carbohydrate content (79.14-81.30%), protein (2.0-2.3%), and crude fiber (4.45-6.34%) content. Fat content was low across all samples (0.60-1.2%), whereas moisture content values ranged from 9.98 to 11.32% with a mean moisture content of approximately 10.65%, indicating good shelf stability. All results were analyzed using descriptive statistics (percentage values), and graphical representations were created using SPSS and Microsoft Excel to visualize differences in microbial and nutritional data. These findings highlight regional differences in microbial and nutritional profiles of the flour, underscoring the need for improved processing practices to enhance safety and quality. This study highlights the nutritional value of plantain flour and recommends quality control measures to ensure consumer health and product integrity.

Keywords: Dietary, Flour, Molecular analysis, Nutritional analysis, Susceptibility profile

INTRODUCTION

Fermentation, a traditional food processing technique has been known over the years to improve the nutritional value, shelf life, and digestibility of its derivative products (Ilelaboye *et al.*, 2021). The Nigerian diet is incomplete without plantain flour, a staple food that has been a part of the country's culinary tradition for centuries (Adepoju and Osunde, 2017). This flour is better for their digestibility, shelf life, and nutritional content when they undergo fermentation (Adeogun *et al.*, 2021). Ogun state has been one of the top producers of plantain flour in Nigeria. The growing global market for gluten-free flours presents Ogun State with an opportunity to expand its export market share and increase revenue from plantain flour. In Ogun State, plantain flour is more than just a basic food (FAO, 2020; Adegunwa *et al.*, 2019). Plantains play a vital role in food security, nutrition, rural livelihoods, and income generation across Africa, the Caribbean, and Latin America. They are culturally significant and serve as staple foods, while also contributing to national economies through local trade and export (Swennen et al., 2013; FAO, 2020). Plantain flour is a staple of Nigerian cuisine. A perfect storm of circumstances has led to its ubiquity on Nigerian tables. Plantains are quite versatile, unlike certain crops that have specific requirements regarding soil type or temperature. Plantain grows well in a variety of environments; it provides a reliable food supply chain even in areas with less fertile ground or erratic rainfall (FAO, 2019). Similar to bananas, plantains are often **inter**cropped with staple crops such as cassava, maize, soybeans, and pumpkins, allowing farmers to harvest multiple products from the same plot and achieve land-equivalent ratios greater than 1.0, indicating

better overall land-use efficiency (Norgrove and Hauser, 2014).

Furthermore, plantain flour provides a surprisingly wide range of culinary options in Nigerian cooking. Plantain flour is also highly versatile, while also being a star of delicious confections like puff puff, which are sugar-dusted, deep-fried dough balls, and it adds a thickening effect to savory soups and stews. In Nigerian kitchens, plantain flour is indispensable due to its capacity to be transformed into a wide variety of delectable foods (Olaoye and Oyewole, 2012). Plantains are cultivated extensively across tropical regions, contributing to their relative affordability compared to other staple commodities (FAO, 2019). Because they are reasonably priced, Nigerians can be assured of access to a reliable and wholesome supply of carbohydrates, which are essential for a balanced diet.

In an oxygen-free (anaerobic) environment, carbohydrates, usually sugars and starches, are fermented by microorganisms such as bacteria and yeast to produce alcohol, acids, or gas. This is essential for the processing of plantains and cassava in several ways. The process of fermentation creates an acidic environment that inhibits the growth of harmful germs, thereby prolonging the shelf life of food (Ray and Sivakumar, 2009). Moreover, the fermentation byproducts, such as alcohol and lactic acid, give food attractive tastes and flavors (Apeh *et al.*, 2021). Certain nutrients may become more bioavailable through fermentation, which facilitates our bodies' ability to absorb them (Marco *et al.*, 2017). The process of fermentation enhances the texture and baking qualities of plantain flour (Unigwe *et al.*, 2023).

Microbial analysis utilizes various techniques to identify, quantify, and classify microorganisms present in food samples. This information is vital for ensuring food safety. Microbial analysis can pinpoint harmful bacteria, viruses, and parasites that can cause foodborne illnesses (Fung *et al.*, 2018). Early detection enables the swift removal of contaminated products from shelves, thereby preventing outbreaks and protecting consumers from potentially severe health issues (Apeh *et al.*, 2021). However, not all microbes are harmful; some can actually accelerate food spoilage. Identifying these spoilage organisms through microbial analysis helps manufacturers optimize storage conditions, implement appropriate handling procedures, and extend the shelf life of food products (Clara, 2023).

Manufacturers can ensure that hygiene requirements are met by identifying potential contamination points and implementing corrective measures through microbiological testing of raw materials, processing environments, and finished products (Fung et al., 2018).

Nutritional analysis examines the nutritional components of our diet, rather than just its appearance. It's a methodical scientific procedure that carefully breaks down food to expose its precise nutritional composition. This breakdown provides the precise quantities of vitamins, minerals, fats, carbohydrates, and protein, in addition to calories. Understanding this is crucial for comprehending the impact of diet on our health (National Research Council, 2025). **This may help** create a diet that provides the body with essential building blocks, such as proteins, vitamins, and minerals, **necessary for growth and maintenance** (National Research Council, 2025).

Fermented plantain flour is a basic food that supply vital nutrients and make a substantial contribution to food security in the study area. However, questions concerning the nutritional value and microbiological safety of these goods are raised by the conventional industrial methods used in their preparation, which are frequently marked by inadequate sanitary conditions. Scientific information about the microbiological composition and nutritional value of plantain flour fermented in Ogun State is scarce, despite its widespread use. Different efforts to enhance the safety and nutritional content of this basic food are hampered by this information gap, bringing about a serious risk to the public's health because these items may contain harmful bacteria. The fermentation process might also result in nutritional loss and the creation of unwanted chemicals while simultaneously improving the flours' sensory qualities. Thus, a thorough analysis of the nutritional and microbiological properties of the fermented plantain flour produced in this area is desperately needed (Oku and Oyadougha, 2024). This study examined fermented plantain flour samples from three senatorial districts in Ogun State for their approximate compositions, such as (moisture, protein, fat, ash, and fiber), determined the particular bacteria involved in the fermentation processing of the plantain

flour, and also screened for the harmful microbes present therein that could pose public health issues.

MATERIALS AND METHODS

Samples Collection

Samples were randomly collected from two commercial markets each across the three senatorial districts in Ogun State, Nigeria. From Ogun Central (The Egbas), samples were obtained from Lafenwa Market in Abeokuta (7°9′29″ N, 3°19′35″ E) and Sayedero Market in Ilaro (6.888° N, 3.014° E). **In** Ogun East **(The Ijebus)**, samples were collected from Obada Market in Ijebu Igbo (6°58′19″ N, 3°59′58″ E) and Imakun Market in Waterside (6°58′19″ N, 3°59′58″ E). From Ogun West (The Yewas), samples were taken from Oju Ore Market in Ota (6.8149° N, 3.1952° E) and Ifo Market in Ifo (6.8149° N, 3.1952° E). A total of six samples were collected and safely transported to the Eco Lab Research Laboratory at Babcock University, Ogun State, for processing and analysis.

Sample Analysis for Bacteria isolation and enumeration

One gram of each sample was aseptically weighed and added to 9 mL of sterile distilled water in a sterile conical flask to form the initial dilution (10 mL). A tenfold serial dilution of the sample was prepared. 1 mL of a dilution factor 10^{-4} suspension was aseptically pipetted and transferred onto a sterile Petri dish. The molten Nutrient Agar was then poured over the inoculum, ensuring the agar and samples were mixed thoroughly by gently swirling the plate. The plates were allowed to solidify and subsequently incubated at 37°C for 24 hours.

Following incubation, the colonies formed on the plates were counted using a colony counter. The total bacterial count was calculated by multiplying the number of colonies by the reciprocal of the dilution factor. The results were expressed as colony-forming units per milliliter (CFU/mL) of the flour sample solution. Plates with 30-300 colonies were considered for enumeration to ensure accuracy and reliability of the results.

Molecular Characterization

The genomic DNA was extracted according to the protocol described in the Quick-DNA miniprep plus kit (Zymo Research, USA). Physiologically young culture samples (200 µL) were added to Eppendorf tubes. An equal volume of biofluid cell buffer was added, and the contents in the tubes were thoroughly vortexed for 10-15 seconds. The tubes were then incubated at 55 ° C for 10 minutes. A volume of Genomic binding buffer (420µL) was added to the digested samples, and they werethoroughly vortexed for 10-15 seconds. The mixtures were transferred to a Zymo-Spin collection tube and centrifuged at ≥ 12000 rpm for 1 minute. Exactly 700 µL of g-DNA wash buffer was added to the spin columns, and they were centrifuged at ≥ 12000 rpm for 1 minute. The collection tubes with the flow-through were discarded. The spin columns were transferred into clean Eppendorf tubes, and exactly 50 µL of DNA elution buffer was added directly to the matrix. The sample was incubated for 5 minutes at room temperature and then centrifuged at maximum speed for 1 minute to elute the DNA. The eluted DNA was used immediately for sanguer sequence identification of the organisms.

PCR Amplification of the 16S r RNA

An aliquot of 2.0 µl of DNA suspension was added to sterile PCR tubes, and a PCR mixture consisting of 12.5 µl of One TaqQuck Load 2X Master Mix Buffer (New England Biolabs) and 0.5 µl of 10 mM 16S primer was added to the PCR tube. The reaction mixture was brought up to 25 µL with sterile water. DNA amplification was carried out using miniPCR (USA) with the following thermal cycling profile: Initial denaturation at 95°C for 2 minutes; 24 cycles of denaturation (30seconds at 95°C), annealing (1 minute at 55°C), and extension (2 minutes at 72°C) and a final extension at 72°Cfor 10 minutes. The digestion was carried out by Agarose gel.

Gel Electrophoresis

Agarose powder, 0.30 g, was measured and dissolved in 25 mL of 1x TEA buffer (1x: 89 mM Tris base, 19 mM boric acid, 2 mM EDTA) solution, stirred, and boiled for 3 minutes. The Agarose solution was allowed to cool to approximately 50 °C, and 5 µL of ethidium bromide was added to the solution, which was then swirled. The solution was poured into a gel tray, and a forming comb was inserted to form wells as the solution solidified. After the gel had solidified, it was transferred into the gel tank and covered with TEA buffer. The amplified DNA and DNA ladder were then loaded into the wells using a pipette that runs from negative to

positive. The gel was left in the tank at 100 volts for 20 minutes, and the bands were observed using a UV-irradiation transilluminator (TMW-20 Trans Illuminator, Alpha Innotech Corporation, USA).

Species Identification

Standard techniques were used to generate unidirectional sequence reads, which were then combined using the Bioedit sequence tool. Molecular Evolutionary Genetics Analysis (MEGA 11) was used to conduct evolutionary analysis.

Antibiotic Susceptibility Profile

The antibiotic susceptibility of the identified bacteria was determined according to the recommendations of the Clinical Laboratory Standard Institute (CLSI, 2021). The antibiotic discs (Diagnostic Tech Gram-negative discs): Ceftriaxone (45 µg), Ofloxacin (5 µg), Amoxicillin-clavulanate (30 µg), Gentamicin (10 µg). Erythromycin, Cloxacillin, and Ceftazidime were also used. A colony of the pure isolate was inoculated into a test tube containing 1 mL of nutrient broth with an incubation period of 16-18 hours at 37°C. The overnight broth was then standardized to match 0.5 McFarland standards. A commercial swab stick was used to inoculate the standardized suspension over the surface of prepared Mueller-Hinton agar plates and incubated at 37°C for 24 h. The apparent zones of inhibition were measured with a meter rule to the adjacent diameter in millimeters.

Determination of Crude Protein

According to the method of Montemurro and Lamagra (2022), 0.5 g of the sample was weighed carefully into a Kjeldahl digestion tube, with the addition of 1 Kjeldahl catalyst tablet and 10 mL of concentrated. H_2SO_4 and was mixed together. The mixture was placed properly in the designated hole of the digestion block heater and left to digest for 4 hours, after which a clear, colorless solution was obtained. The digest was cooled and carefully transferred into 100ml volumetric flask and distilled water was added. A 5ml portion of the digested sample was pipetted into the body of the already steamed Markham distillation apparatus via the small funnel aperture.

Additionally, 5ml of 40% (w/v) NaOH was added through the small funnel aperture. The mixture was steam-distilled for 2 minutes into a 50 mL conical flask containing 10 mL of a 2% boric acid solution with a mixed indicator, placed at the receiving tip of the condenser. The Boric Acid plus indicator solution changes colour from red to green showing that all the ammonia liberated have been trapped.

The green-colored solution obtained was then titrated against 0.01 N HCl contained in a 50 mL buret. At the endpoint or equivalent point, the green colour turns to a wine colour, indicating that all the Nitrogen trapped as Ammonium Borate [$(NH4)_2BO_3$] has been removed as Ammonium chloride (NH_4Cl).

The percentage nitrogen in this analysis was calculated using the formula:

% N = (Titre value x Atomic mass of Nitrogen x Normality of HCL used x 4)/

N x Volume of flask containing the digest x 100

1

Weight of sample digested in milligram x Vol. of digest for steam distillation. The crude protein content is determined by multiplying the percentage of Nitrogen by a constant factor of 6.25, i.e., % CP = % N × 6.25.

Determination of Crude Fat

Using FSSAI (2022) methods, 1 g of the dried sample was weighed into a fat-free extraction thimble and lightly plugged with cotton wool. A 250 ml Soxhlet flask, which had been previously dried in the oven, cooled in a desiccator, and weighed, was placed in the extractor with the thimble and fitted with a reflux condenser. The Soxhlet flask was then filled to three-quarters of its volume with petroleum ether (bp 40°C – 60°C). The heater was operated for 6 hours with constant running water from the tap to facilitate condensation of the ether vapor. The extraction process was constantly monitored for ether leaks, and the heat source was adjusted accordingly to ensure the ether boiled gently. The ether was left to siphon over several times until it was short of siphoning. Immediately after the extraction process, the thimble containing the sample was removed, and the ether content was drained into an ether stock bottle. The Soxhlet flask was also removed from the extraction machine, and the ether content was carefully drained until it was ensured that the Soxhlet flask contained only the fat and oil.

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

The Soxhlet flask, which contained the fat and oil, was dried to a constant weight in the oven, cooled in a desiccator, and weighed. If the initial weight of dry Soxhlet flask is W_0 and the final weight of the oven-dried flask + fat/oil is W_1, the percentage fat/oil is obtained by the formula:

$$\frac{W_1 - W_0 \times 100}{\text{wt of sample} \quad 1}$$

Determination of Crude Fiber

According to the FSSAI (2022), 2.0 g of the sample was accurately weighed into the fibre flask, and 100 mL of 0.255 N H_2SO_4 was added. The mixture was heated under reflux for 1 hour with the heating mantle. The hot mixture was filtered through a fibre sieve cloth; the filtrate obtained was discarded, and the residue was returned to the fibre flask. To this, 100 ml of 0.313 N NaOH was added, and the mixture was heated under reflux for another hour. The mixture was filtered through a fibre sieve cloth, and 10 mL of acetone was added to dissolve any organic constituents. The residue was washed with 50 mL of hot water on the sieve cloth before being finally transferred into the crucible. The crucible and residue were oven-dried at 105^0C overnight to drive off moisture. The oven-dried crucible containing the residue was cooled in a desiccator and later weighed to obtain the weight W_1. The crucible with weight W_1 was transferred to the muffle furnace to obtain the ash at 550^0C for 4 hours.

The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccator and weighed to obtain W_2. The difference (W_1 – W_2) gives the weight of fibre.

The percentage fibre is obtained by the formula:

$$\% \text{ fibre} = \frac{(W_1 - W_2) \times 100}{\text{wt of sample}}$$

Determination of Ash

According to the FSSAI (2022), 2.0 g of the sample was weighed into a porcelain crucible. This was transferred into the muffle furnace set at 550 °C and left for about 4 hours; at this time, it had turned to white ash. The crucible and its contents were cooled to approximately 100 °C in air, then cooled to room temperature in a dessicator and weighed.

The percentage ash was calculated from the formula below:

$$\text{Ash content} = \frac{\text{wt. of ash} \times 100}{\text{Original wt.} \quad 1}$$

of sample

Determination of Dry Matter and Moisture Content

According to the FSSAI (2022), 2g of the sample was weighed into a previously weighed crucible. The crucible plus sample taken was then transferred into the oven set at 100 °C to dry to a constant weight overnight. At the end of the 24 hours, the crucible with the sample was removed from the oven and transferred to a dessicator, cooled for 10 minutes, and weighed.

If the weight of the empty crucible is Wo

weight of crucible plus sample is W_1

weight of crucible plus oven-dried sample W_3

$$(\%\ DM)\ \%\ \text{Dry Matter} = \frac{W_3 - Wo \times 100}{W_1 - Wo \quad 1}$$

$$\%\ \text{Moisture} = \frac{W_1 - W_3 \times 100}{W_1 - Wo \quad 1}$$

or % Moisture = 100 – % DM.

Statistical Analysis

The data generated were analyzed using descriptive statistics (expressed as percentage values) for graph plotting in IBM SPSS and Microsoft Excel.

RESULTS

The total bacterial counts (TBCs) of plantain flour samples collected from six locations across Ogun State showed varying microbial loads ranging from 1.8×10^4 CFU/ml (Ilaro) to 3.5×10^4 CFU/ml (Waterside) with a mean TBC of 2.73×10^4 CFU/ml. The standard deviation was 0.61×10^4 CFU/ml, indicating moderate variability in microbial loads across locations (Fig. 1). The highest TBC observed in Waterside may indicate poorer handling or environmental conditions, while the lowest in Ilaro could reflect better hygiene or storage practices.

The gel electrophoresis analysis of the PCR-amplified 16S rRNA gene fragments revealed

distinct DNA bands corresponding to expected amplicon sizes. A total of 19 lanes were visualized, with Lane 1 containing the molecular weight marker (DNA ladder), which served as a size reference. The ladder displayed well-resolved bands, allowing for accurate estimation of DNA fragment sizes in the adjacent sample lanes. Most of the bacterial DNA samples produced single, prominent bands within the size range of 1,400 to 1,500 base pairs (bp), consistent with the expected length of the 16S rRNA gene targeted by the universal primers. The migration distances of these bands were inversely proportional to their molecular sizes, fragments closer to 1,500 bp migrated shorter distances compared to smaller DNA fragments. The clear and specific amplification patterns confirm the successful isolation and amplification of bacterial 16S rRNA genes suitable for sequencing and subsequent identification (Fig. 2).

Molecular characterization of the isolates revealed the presence Lysinibacillus spp., *Providencia rettgeri, Bacillus licheniformis, Bacillus thuringiensis, Bacillus pseudomycoides,* Pusillimonas spp. and *Bacillus tropicus* with percentage similarities of (98.7-99.8)% with those deposited in the gene bank upon blasting (Table 1). The identities of the isolates were confirmed using 16S rRNA gene sequencing. Chromatograms of the DNA sequences were analyzed and matched against the NCBI database for confirmation.

The statistical analysis revealed that 5 of 8 antibiotics (62.5%) had 100% resistance rates among isolates. Ofloxacin was the most effective antibiotic, showing activity against 5 of the 7 isolates. Multidrug resistance was particularly evident in *Bacillus tropicus* and *Lysinibacillus spp.*, while *Pusillimonas spp.* showed the lowest resistance (Fig.3, Table 2). These findings underscore the need for regular surveillance and judicious antibiotic use in food production to curb the spread of antimicrobial resistance.

Nutritional analysis of the plantain flour samples revealed consistent but regionally variable nutrient profiles across the six sampling locations. The mean moisture content was approximately **10.65%**, with values ranging from 9.98% (Ado-Odo-Ota) to 11.32% (Ilaro), indicating generally good shelf-stability. Crude fiber content averaged 5.23%, highest in Ilaro (6.34%) and lowest in Ado-Odo-Ota (4.45%), suggesting potential digestive benefits with regional variations likely due to processing or cultivar differences. Fat content remained low across all samples, averaging 0.88%, with Ado-Odo-Ota showing the highest value (1.20%), reaffirming plantain flour as a low-fat carbohydrate source. Ash content, a proxy for mineral presence, varied more widely (0.30%-1.50%) with an average of 0.67%, indicating potential differences in soil mineral content or handling methods. The protein content was modest and stable, with a mean of 2.12%, indicating that plantain flour provides only limited protein unless fortified. Carbohydrate content was the most abundant nutrient, averaging 80.46%, with a range of 79.14% (Ilaro) to 81.30% (Abeokuta), highlighting its value as an energy-dense food (Tables 3 and 4).

Overall, the flour exhibited desirable properties, including low fat, moderate fiber, and high carbohydrate levels, which support its use as a staple energy source. The observed variability in nutritional content may be attributed to differences in soil composition, plantain variety, and processing techniques.

Table 1: Percentage Identity and GenBank Accession Numbers of Bacterial Isolates Identified by 16S rRNA Gene Sequencing

Isolate Code	Species Identified	% Identity	GenBank Accession No.
JD19	Lysinibacillus spp.	99.8%	MH628167.1
AP8	*Providencia rettgeri*	99.1%	OP132978.1
AP4	*Bacillus licheniformis*	99.9%	OP164283.1
JD14	*Bacillus thuringiensis*	99.6%	MT510408.1
JD15	*Bacillus pseudomycoides*	98.9%	KY344820.1
OU5	*Bacillus tropicus*	99.4%	CP119875.1
OU8	Pusillimonas spp.	98.7%	MK418887.1

E-ISSN: 2814 – 1822; P-ISSN: 2616 – 0668

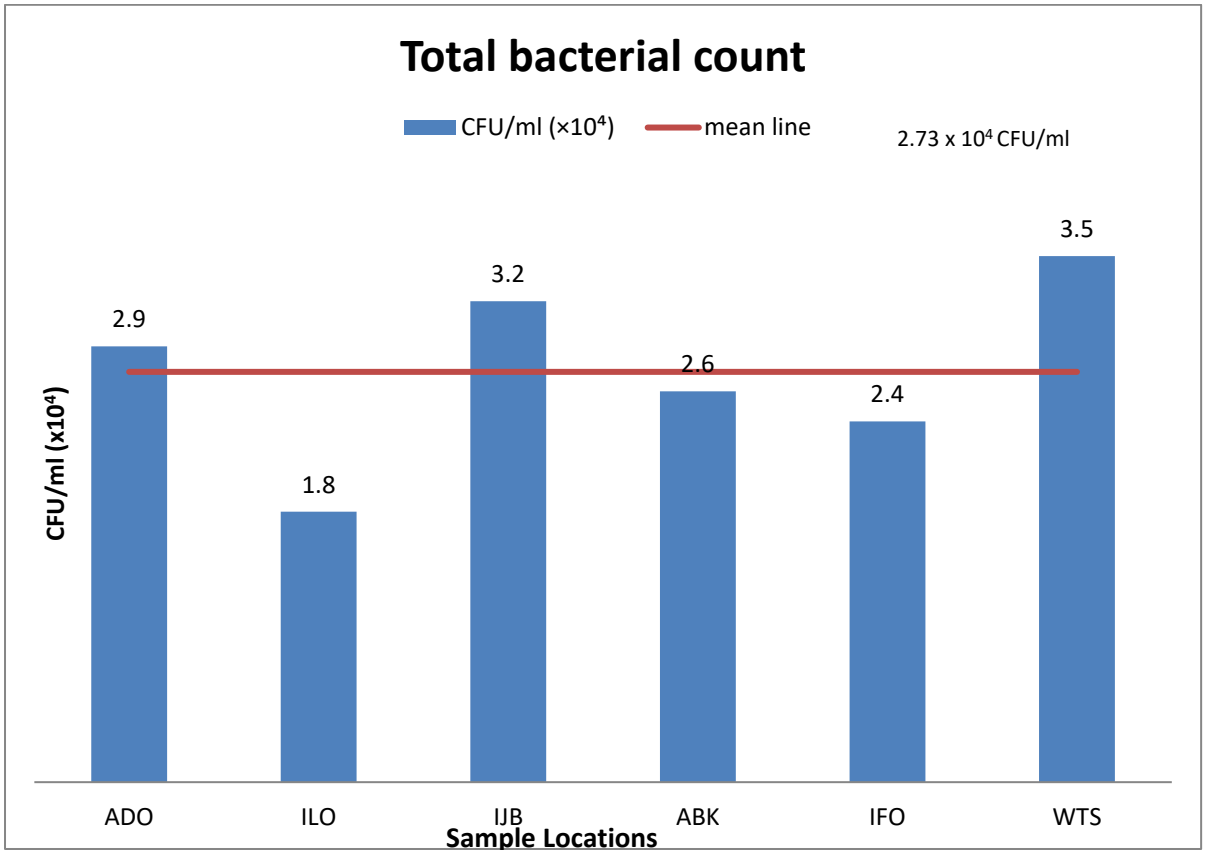

Fig. 1: Total bacterial count (CFU/ml ×10⁴) from plantain flour across six sample locations in Ogun State, Nigeria.

Table 2: Antibiotic Susceptibility of the Isolates.

Isolates	AUG (mm)	OFX (mm)	CAZ (mm)	CRX (mm)	GEN (mm)	CTR (mm)	ERY (mm)	CXC (mm)
Bacillus licheniformis	11	9	12	10	11	9	12	10
Bacillus thuringinesis	13	21	10	11	11	7	9	6
Pusilliomonas spp.	14	17	9	15	22	10	17	7
Providencia rettgeri	9	22	13	12	17	15	9	10
Lysinibacillus spp.	10	16	15	8	8	12	7	8
Bacillus tropicus	7	12	8	7	12	14	6	6
Bacillus pseudomycoides	11	18	11	9	14	8	8	6

CXC: Cloxacillin, **ERY:** Erythromycin, **CTR:** Ceftriaxone, **GN:** Gentamicin, **CRX:** Cefuroxime, **CAZ:** Ceftazidime, **OFX:** Ofloxacin, **AUG:** Amoxicillin/Clavulanic Acid, **CLSI** = Clinical and Laboratory Standards Institute

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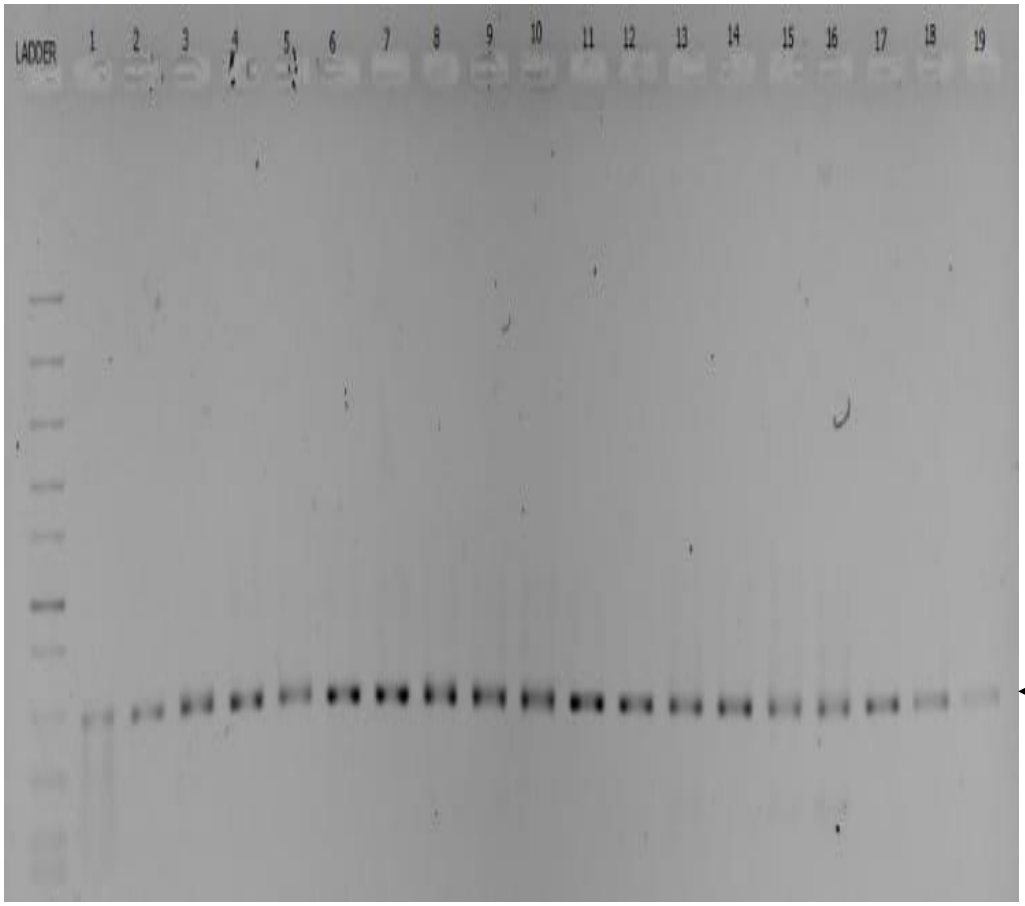
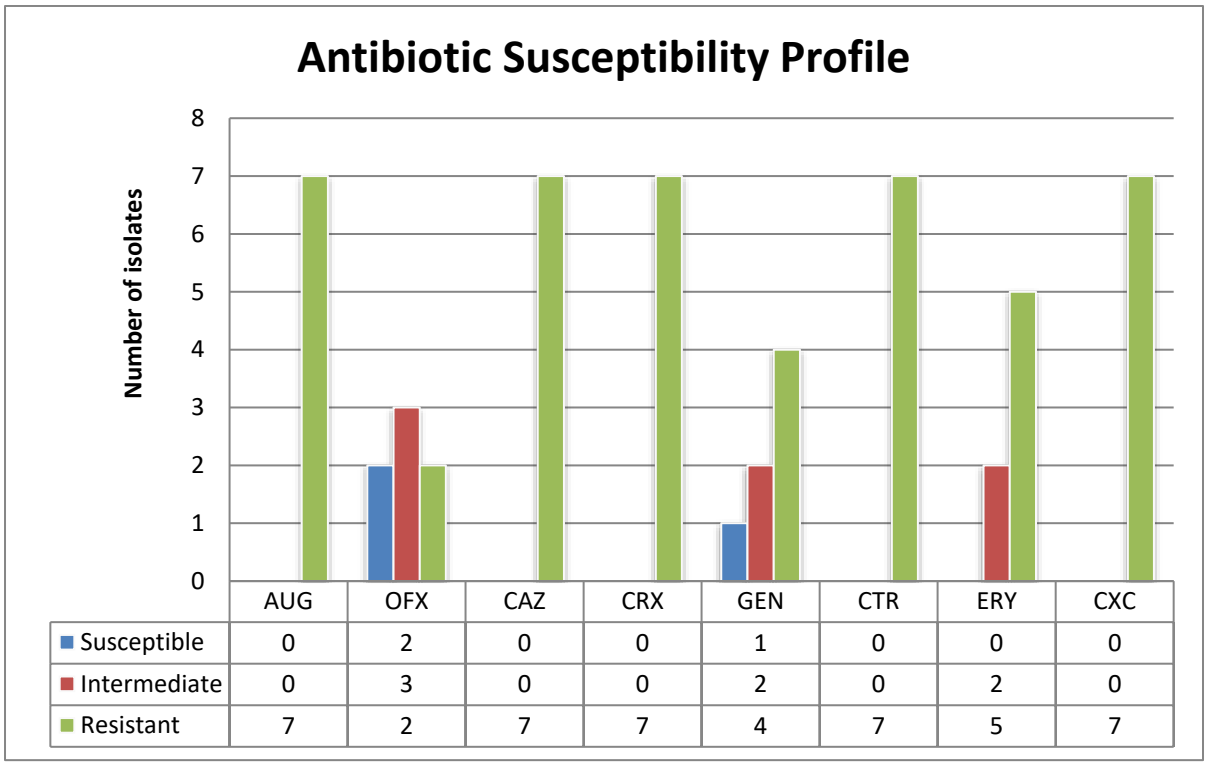


Fig.2: Gel Electrophoresis of Isolated Bacteria



	AUG	OFX	CAZ	CRX	GEN	CTR	ERY	CXC
Susceptible	0	2	0	0	1	0	0	0
Intermediate	0	3	0	0	2	0	2	0
Resistant	7	2	7	7	4	7	5	7

Fig.3: Antibiotic susceptibility profile using the Clinical Laboratory Standard Institute (CLSI) chart (S= ≥21, I= 16-20, R= ≤15). S= Sensitive, I=Intermediate, R= Resistant. CLSI (mm) S= ≥21 I= 16-20 R= ≤15

Table 3: Percentage mean and standard deviation of Nutritional Contents of the Plantain Flour Samples

Nutrient	Mean (%)	SD	Min (%)	Max (%)
Moisture	10.65	0.46	9.98	11.32
Crude Fibre	5.23	0.63	4.45	6.34
Fat	0.88	0.20	0.60	1.20
Ash	0.67	0.43	0.30	1.50
Protein	2.12	0.11	2.00	2.30
Carbohydrate	80.46	0.69	79.14	81.30

Table 4: Nutritional Contents of the Plantain Flour Samples

Sample Location	Moisture Content (%)	Crude Fibre (%)	Fat (%)	Ash content (%)	Protein content (%)	Carbohydrate (%)
IFO	11.05	5.12	0.90	0.30	2.2	80.43
ILO	11.32	6.34	0.60	0.50	2.1	79.14
WTS	10.90	4.85	0.80	0.40	2.3	80.75
ADO	9.98	4.45	1.20	1.5	2.0	80.87
ABK	10.25	5.00	0.75	0.6	2.1	81.30
IJB	10.40	5.65	1.0	0.7	2.0	80.25

IFO- Ifo, **ILO-** Ilaro, **WTS-** Waterside, **ADO-** Ado Odo, **ABK-** Abeokuta, **IJB-** Ijebu Igbo

DISCUSSION

The analysis revealed substantial differences in microbial load and nutritional content across the regions, likely influenced by environmental conditions, hygiene standards, and processing methods.

Proximate analysis showed slight regional variation in the nutritional composition of plantain flour across Ogun State, consistent with environmental and agricultural differences. Moisture content ranged from 9.98% in Ado-Odo-Ota to 11.32% in Ilaro. These findings align with those of Olaleye et al. (2021), who reported moisture levels between 9.5% and 12%, supporting the shelf stability of plantain flour compared to more perishable products, such as cassava flour.

Fiber content ranged from 4.45% in Ado-Odo-Ota to 6.34% in Ilaro, supporting previous findings by Akinmoladun *et al.* (2020), who reported values between 4.5% and 7%. High fiber content is nutritionally beneficial, promoting digestive health. The fat content was low, ranging from 0.60% in Ilaro to 1.2% in Ado-Odo-Ota, which aligns with Ibrahim et al. (2021), who reported a range of 0.5–1.5%. The low fat profile confirms plantain flour's value as a carbohydrate-rich, low-fat food source.

Ash content, which reflects mineral composition, ranged from 0.30% in Ifo to 1.50% in Abeokuta, in line with Agboola *et al.* (2019), who found values of 0.4–1.8%. Variations may be attributed to soil mineral content and post-harvest handling. Protein content ranged from 2.0% in Ifo to 2.3% in Ilaro, consistent with Akinmoladun *et al.* (2020), who reported 2.0–2.5%. While modest, the protein content suggests potential nutritional benefits in plant-based diets, especially in regions at risk of malnutrition.

The carbohydrate content was high, ranging from 79.14% in Ilaro to 81.30% in Abeokuta, making plantain flour an excellent source of energy. These results are comparable to those of Ogundare et al. (2020), who reported carbohydrate levels of 79–83%. Studies by Oluwalana *et al.* (2021) and Babatunde *et al.* (2020) also confirmed high carbohydrate content and moderate protein and fiber levels in plantain flour across Southwest Nigeria.

Overall, the nutritional profile of plantain flour in this study supports its role as a healthy, energy-rich food product. Regional variations observed may be attributed to differences in soil quality, processing techniques, and environmental conditions (Olowe *et al.*, 2018; Akinmoladun *et al.*, 2020).

The total bacterial count (TBC) in the plantain flour samples ranged from 1.8×10^4 CFU/mL in Ilaro to 3.5×10^4 CFU/mL in Waterside, with a mean TBC of 2.73×10^4 CFU/mL. These values are consistent with those reported by Amoako et

al. (2022), who found 2.3 × 10^4 to 4.0 × 10^4 CFU/ml in Ghana. However, Oku and Oyadougha (2024) recorded lower TBCs in packaged plantain flour (4.52 × 10^2 CFU/g) compared to unpackaged (6.01 × 10^2 CFU/g), highlighting packaging as a key factor in microbial safety. In comparison, Molapo *et al.* (2020) observed lower counts in South African samples, whereas Nguefack et al. (2020) reported higher levels in Kenyan samples, which they attributed to the use of contaminated water in processing. These findings underscore that inadequate packaging and hygiene practices in Ogun State may be responsible for elevated microbial loads.

Using molecular identification techniques, a limited number of bacterial isolates were identified, including *Bacillus licheniformis*, *Bacillus tropicus*, and *Lysinibacillus* spp. Notably, samples from Ijebu-Igbo and Ifo yielded no detectable isolates, possibly due to reduced contamination, differences in moisture content, or improved handling. The prevalence of *Bacillus* species aligns with their known roles in fermented foods, contributing enzymatic activities that enhance nutritional quality and shelf life (Sindhu *et al.*, 2021; Suleiman *et al.*, 2022). Moreover, these species exhibit antimicrobial properties (Lai *et al.*, 2018), supporting food preservation. However, the detection of *Providencia rettgeri*, an opportunistic pathogen, indicates potential health risks due to unhygienic handling (Adeyemi *et al.*, 2021). Other isolates such as *Pusillimonas* spp. and *Bacillus thuringiensis*, though typically non-pathogenic, further highlight the need for stringent quality control and environmental monitoring (Santos *et al.*, 2020).

The bacterial profiles identified in this study differed from others. For example, Amoako *et al.* (2022) reported the presence of *Bacillus cereus* and *Bacillus subtilis* using metagenomics, whereas Nguefack *et al.* (2019) employed culture-based methods and identified *Enterobacter* and *Lactobacillus species*, which were not detected in this study. This suggests that culture-independent molecular approaches, such as PCR and 16S rRNA sequencing used here and by Mwangi *et al.* (2021), offer improved sensitivity for identifying less culturable bacteria. Eze *et al.* (2022) reported similar isolates in Southern Nigeria, aligning with findings from Abeokuta and Ado-Odo-Ota. Ogunbanjo *et al.* (2019), using traditional methods in Ogun State, also found *Bacillus* spp.; however, B. cereus was absent, likely due to methodological differences.

Antibiotic susceptibility testing revealed significant resistance among the isolates. *Bacillus licheniformis* and *B. thuringiensis* were only susceptible to Ofloxacin (22 mm and 21 mm, respectively), and resistant to Augmentin, Gentamicin, and Ceftazidime. *Pusillimonas* spp. showed intermediate susceptibility to Ofloxacin (17 mm) and Gentamicin (22 mm), while *Providencia rettgeri* was susceptible to Ofloxacin (22 mm), intermediate to Gentamicin (17 mm), and resistant to others. Other isolates, such as *Lysinibacillus* spp. and *B. tropicus*, exhibited widespread resistance.

These results align with those of Oladimeji et al. (2020), who reported greater than 85% resistance to ß-lactams among *Bacillus* isolates. Eze *et al.* (2021) also reported high susceptibility of *B. thuringiensis* to Ofloxacin (21–24 mm). Similarly, Ogunbanjo *et al.* (2019) documented *Providencia* spp. susceptibility to Ofloxacin (20–23 mm) and intermediate Gentamicin resistance (16–18 mm). However, discrepancies exist with Okonkwo *et al.* (2020), who found *B. licheniformis* to be susceptible to Gentamicin (20–23 mm), whereas this study reported resistance (11 mm). Adebayo *et al.* (2019) reported *Lysinibacillus* susceptibility to Augmentin and Ceftriaxone (22–24 mm), contrary to resistance observed here. Ibrahim *et al.* (2020) and Adediran *et al.* (2021) similarly confirmed the efficacy of Ofloxacin but noted resistance to macrolides and Augmentin, respectively.

Overall, the observed patterns indicate the emergence of multidrug resistance in foodborne bacteria, raising concerns about food safety. While Ofloxacin remains a viable treatment option, resistance to common antibiotics necessitates robust antimicrobial stewardship and routine surveillance to mitigate public health risks.

CONCLUSION

This study highlights the significant microbial diversity, nutritional richness, and concerns regarding antibiotic resistance present in fermented plantain flour sourced from various markets in Ogun State, Nigeria. The variations in microbial load and nutrient composition across locations underscore the influence of environmental and processing practices on the final product quality. The dominance of *Bacillus* spp., known for their fermentation-enhancing properties, suggests a potential role in both preserving and maintaining the functional

quality of plantain flour. However, the detection of antibiotic-resistant strains calls attention to public health risks associated with inadequate food handling and uncontrolled antibiotic use in food production systems.

Despite its high carbohydrate content, plantain flour offers promising health benefits when processed safely, particularly when enriched to improve its protein and fiber content. The findings of this study emphasize the need for improved handling, enhanced fermentation techniques, and standardized production methods to ensure consistent nutritional value and microbial safety. Introducing controlled fermentation processes, such as the use of starter cultures and regulated drying methods, could minimize contamination and maximize the beneficial attributes of the flour.

ACKNOWLEDGMENT

We wish to express our gratitude to God Almighty for guiding us through this study, and also appreciate the management and staff of Abraham Adesanya Polytechnic for providing a conducive learning environment.

CONFLICT OF INTEREST

There was no conflict of interest among the authors

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