

## EVALUATION OF BACTERIAL CONTAMINATION IN *Pomadasys Jubelini*: IMPACT OF PROCESSING METHODS FROM LAGOS LAGOON COMMUNITY

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### ABSTRACT

This study aimed to investigate the microbial load and diversity of bacterial species in the internal tissues of Grunter fish (*Pomadasys jubelini*) sampled from Lagos Lagoon. Twenty-five (25) fish samples of *Pomadasys jubelini* were aseptically collected, weighed, and measured with care. Fresh, frozen, fried, roasted, and boiled samples were then analyzed using standard microbiological techniques. The microbial load varied across the sample types: frozen samples had counts ranging from  $0.9$  to  $1.3 \times 10^8$  cfu/g, fresh samples ranged from  $8.1$  to  $9.9 \times 10^8$  cfu/g, fried samples showed  $0.1$  to  $0.2 \times 10^8$  cfu/g, roasted samples ranged from  $0.4$  to  $0.6 \times 10^8$  cfu/g, and boiled samples had counts between  $0.8$  and  $1.0 \times 10^8$  cfu/g. The highest bacterial count was found in fresh samples ( $9.9 \times 10^8$  cfu/g), while the lowest was in fried samples ( $2.0 \times 10^8$  cfu/g). There was significant variation in the bacterial diversity with a predominance of gram-negative bacteria among the isolates. The presence of potential pathogenic bacteria in the internal tissues of these processed fish raises public health concerns, highlighting the need for strict hygienic practices during the handling and processing of fish sourced from brackish waters.

**Keywords:** Microbial, bacterial, processed fish, fish samples, Lagos.

### INTRODUCTION

The grunter fish, *Pomadasys jubelini*, belongs to the family *Pomadasysidae* and is significant in the ecology and fisheries of West Africa and other inland waters (Salama, 2024). *Pomadasys jubelini* plays a vital role in the riparian communities of the Lagos Lagoon, valued for its year-round abundance, flavorful flesh, and significant economic and nutritional contributions. However, as a key species targeted by artisanal fisheries, it is now in decline in Nigeria due to the combined impacts of overfishing, habitat degradation, and pollution (Olopade, 2020). It serves as a valuable source of nutritious protein, providing essential nutrients for the body (Amosu, 2023). Within this family, three species are recognized: *Pomadasys perotiti*, *P. rogeri*, and *P. jubelini*, the latter of which can be found in Lagos Lagoon (Adebiyi, 2013).

Fish is a crucial source of low-fat protein and Omega-3 fatty acids, vital for both infants and adults (Simopoulos, 2020). As a key food staple, fish has been consumed since prehistoric times and continues to be integral to diets worldwide, supporting livelihoods through harvesting, processing, and distribution (Hasselberg, 2020). However, fish, including finfish and shellfish, are highly susceptible to microbial contamination due to their soft tissues and aquatic environments (Suresh, 2022). The microbial flora found in fish often reflects the quality of the waters they inhabit, with sewage-polluted waters posing a significant risk of

introducing pathogenic microorganisms (Codello, 2021).

Once a fish dies, its immune system ceases to function, allowing bacteria to invade and proliferate in the flesh, which leads to spoilage. Microorganisms are the primary cause of seafood spoilage, with specific spoilage organisms (SSOs) producing off-flavors that make fish unfit for consumption (Luqman, 2024). Contaminated fish can result in foodborne illnesses, including food poisoning, bacillary dysentery, and typhoid (Parlapani, 2023), making the study of microbial contamination in fish an important area of research. Some bacterial isolates may require advanced identification methods, such as 16s rDNA sequencing with universal primers, as phenotypic characteristics alone may not suffice (Dabban, 2024)

Understanding the dynamics of microbial contamination in fish especially how different processing methods influence microbial load and diversity is essential for enhancing food safety (Mahunu, 2024). This study aims to explore these factors, focusing on bacterial contamination during various processing methods to mitigate public health risks.

### MATERIALS AND METHODS

A total of five samples of Grunter fish were assigned to each processing method. Twenty-five (25) Grunter fish (*Pomadasys jubelini*) samples were randomly selected and purchased from Makoko

Market in Lagos. The samples were immediately placed in a sterile cooler packed with ice and transported to the Microbiological Laboratory at the University of Lagos for analysis.

#### **Morphometric Parameters**

Morphometric parameters of the fish samples, including weight, total length, and standard length, were measured using a top-loading balance (Reshy Balance) for weight and a graduated measuring ruler for length. Measurements were recorded to two decimal places (Alabi *et al.*, 2008).

#### **Processing of Fish Samples**

The fish samples underwent several processing methods on their internal surfaces, including freezing, boiling, frying, and drying. The samples were organized based on the number of processing stages to be applied. The procedures are detailed below:

##### **Freezing**

The fish samples were stored in a freezer for 24 hours at 0°C. After this period, the samples were thawed, and excess water was removed using a clean cloth prior to bacterial analysis.

**Drying:** The fish samples were gutted, and the viscera were removed before being washed with clean water. The cleaned fish were then placed in a sterilized microwave oven and dried at 200°C for 1 hour. After drying, the samples were allowed to cool before bacterial analysis was conducted.

##### **Boiling Method**

The fish sample was gutted, and the visceral organs were removed, followed by a thorough wash with clean water. The cleaned fish was placed in a sanitized pot with 10 cm<sup>3</sup> of water and cooked over camp gas at 100°C for 20 minutes. After boiling, the fish was allowed to cool before conducting bacterial analysis.

##### **Frying Method**

The fish sample was gutted and washed with clean water. A clean frying pan was placed on camp gas, and 0.5 L of groundnut oil (Power Oil) was added, heating it to 100°C. Once the oil was hot, the fish sample was fried for 10 minutes, then removed from the oil and allowed to cool before bacterial analysis.

##### **Sample Preparation**

After cooking, samples were aseptically dissected to remove the skin, and 1 g of tissue was weighed using a top-loading balance. The tissue was then gently mashed with a pestle and mortar. All glassware used

was sterilized in an autoclave at 160°C for 90 minutes.

#### **Preparation of Stock Culture**

A section from each of the processed fish samples was carefully removed using sterile scalpels, scissors, and razor blades, and placed into sterile Petri dishes. Four grams of each sample were then homogenized using a mortar and pestle to achieve a uniform distribution of cells for the stock culture.

#### **Amplification of 16S rDNA and Sequencing**

The 16S ribosomal DNA (rDNA) region of the bacterial isolates was amplified by PCR using the universal primers 27F and 1492R. The resulting PCR products were then sequenced, and sequence analysis was performed using the Basic Local Alignment Search Tool (BLAST) to identify the bacterial species based on sequence similarity.

#### **Statistical Analysis**

All morphometric data and bacterial counts were analyzed statistically using SPSS. Regression analysis was conducted to determine the Length-Weight Relationship (LWR), while ANOVA was used to compare bacterial loads across different processing methods. Correlation tests were performed to assess the relationship between condition factors and bacterial load. A significance level of  $p < 0.05$  was set for all statistical tests.

#### **RESULTS**

The total bacterial count, expressed as colony-forming units per gram (CFU/g), was assessed from the internal tissue of *Pomadasys jubelini* across various treatment methods, including fresh, frozen, fried, roasted, and boiled samples. Significant variations in bacterial loads were observed depending on the preparation method. Fresh samples exhibited the highest bacterial counts, while fried samples demonstrated the lowest (Table 1). The mean number of bacterial colonies was 90, resulting in a total bacterial count of  $9.0 \times 10^8$  CFU/g and a log CFU/g value of 9.0.

The frozen samples showed a significant reduction in bacterial colonies, with a mean of 11 colonies and a total count of  $1.1 \times 10^8$  CFU/g (log CFU/g = 8.0). The fried samples exhibited the lowest bacterial count, with only 2 colonies ( $0.2 \times 10^8$  CFU/g) and a log CFU/g value of 7.3. The roasted samples had a mean of 9 colonies, translating to a total count of  $0.9 \times 10^8$  CFU/g and a log CFU/g value of 8.0. Finally, the boiled samples showed 5 colonies, resulting in a total bacterial count of  $0.5 \times 10^8$  CFU/g and a log CFU/g of 7.7.

**Table 1: Total Bacterial Count from Internal Tissue of *Pomadasys jubelini***

Sample	Mean Number of Colonies	Total Colony Count (CFU/g)	Log CFU/g
Fresh	90	$9.0 \times 10^8$	9.0
Frozen	11	$1.1 \times 10^8$	8.0
Fried	2	$0.2 \times 10^8$	7.3
Roasted	9	$0.9 \times 10^8$	8.0
Boiled	5	$0.5 \times 10^8$	7.7

**Note:** Colony count represent the mean of replicate measurements

The ANOVA test was used to analyze the significant differences in bacterial counts across preparation methods. The test revealed that the preparation method significantly influenced bacterial loads ( $F(4, 10) = 13,275.0, p < 0.001$ ). Tukey's HSD post-

hoc analysis identified statistically significant differences in bacterial counts for most comparisons, except between boiled and fried samples ( $p = 0.123$ ) (Table 2).

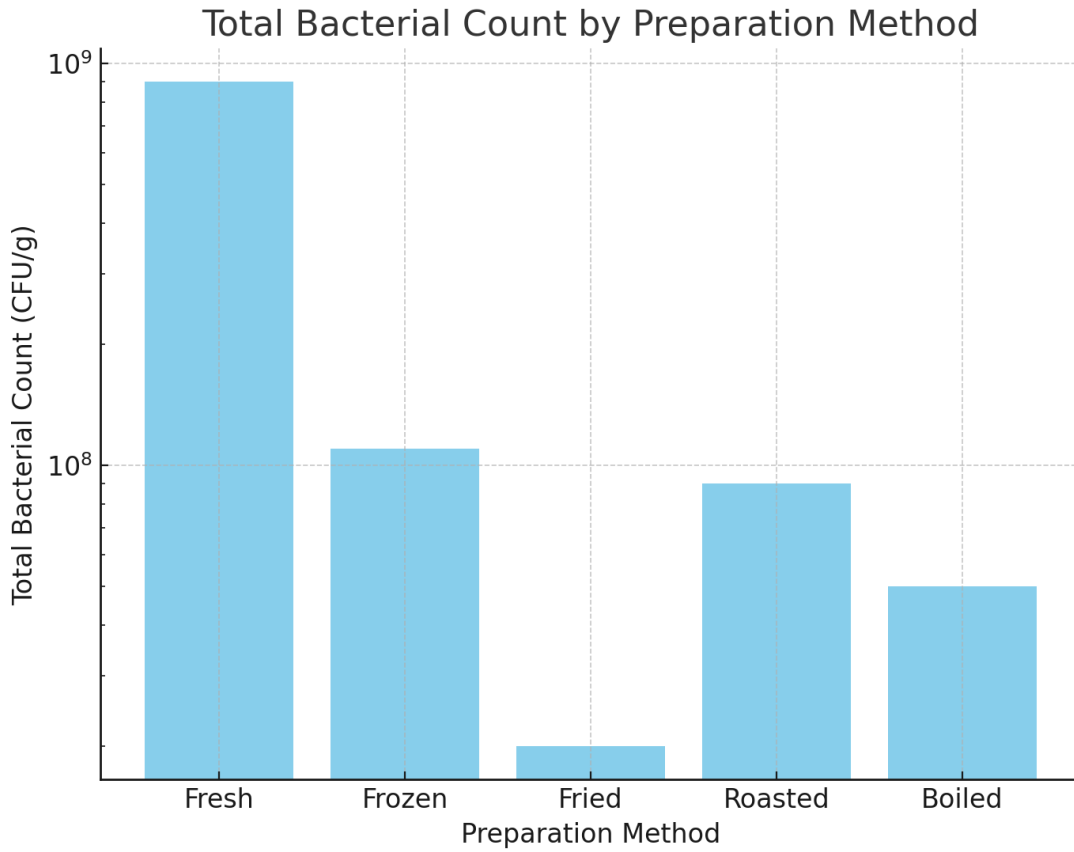
**Table 2: Statistical Significance Comparisons of Bacteria Counts Across Preparation Methods**

Comparison	Mean Difference (CFU/g)	p-value
Boiled vs. Fresh	$-8.50 \times 10^8$	< 0.001
Boiled vs. Fried	$3.00 \times 10^7$	0.123
Boiled vs. Frozen	$-6.00 \times 10^7$	< 0.001
Boiled vs. Roasted	$-4.00 \times 10^8$	< 0.001
Fresh vs. Fried	$8.80 \times 10^8$	< 0.001
Fresh vs. Frozen	$7.90 \times 10^8$	< 0.001
Fresh vs. Roasted	$5.00 \times 10^8$	< 0.001
Fried vs. Frozen	$-8.50 \times 10^7$	< 0.001
Fried vs. Roasted	$-3.80 \times 10^8$	< 0.001
Frozen vs. Roasted	$-2.90 \times 10^8$	< 0.001

Significant at  $P < 0.05$

The total bacterial count (CFU/g) was compared across five preparation methods: Fresh, Frozen, Fried, Roasted, and Boiled (Fig. 1). The bar chart illustrates that Fresh samples had the highest bacterial load, averaging  $9.0 \times 10^8$  CFU/g. In contrast, Frozen samples showed a significant

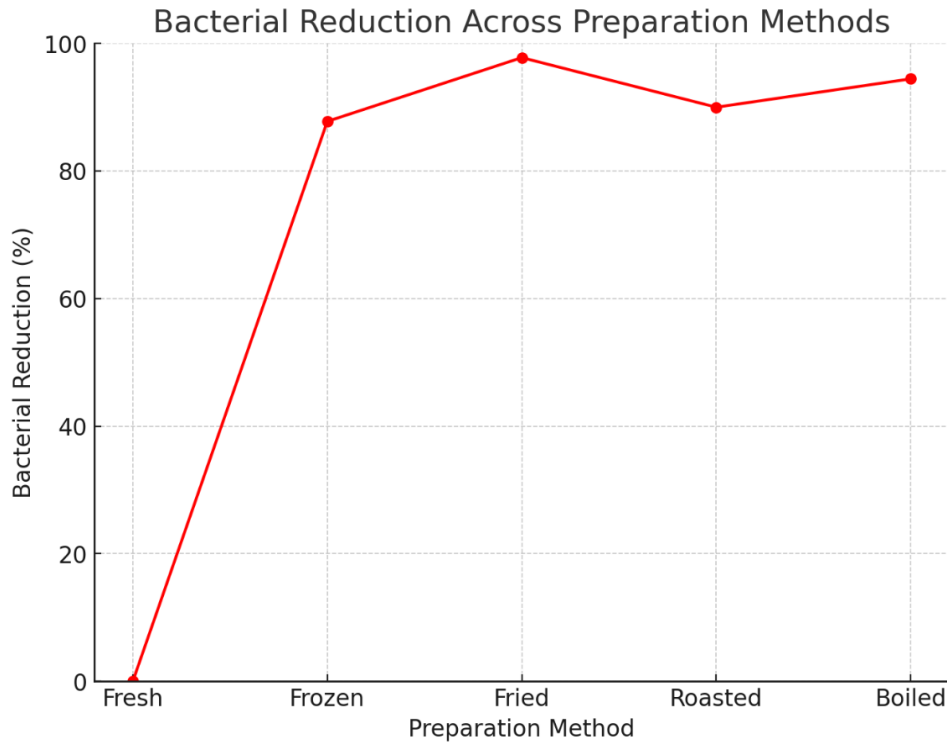
reduction in bacterial load, at  $1.1 \times 10^8$  CFU/g. Heat-treated methods—Fried, Roasted, and Boiled—exhibited the lowest bacterial counts, with Fried samples demonstrating the most substantial reduction at  $0.2 \times 10^8$  CFU/g.



**Fig. 1: Bar Chart Showing Total Bacterial Count (CFU/g) by Preparation Method**

The percentage reduction in bacterial load, relative to the fresh sample as the baseline (0% reduction), was evaluated across different treatments. Frozen samples exhibited an 87.8% reduction in bacterial load. Among the heat treatments, frying was the most effective, achieving a 97.8% reduction,

followed by boiling with a 94.4% decrease, and roasting, which reduced the bacterial load by 55.6%. These results, highlight the effectiveness of cooking methods in significantly lowering bacterial levels, with frying emerging as the most efficient (Fig. 2).



**Fig. 2: Line Graph Showing Bacterial Reduction Across Preparation Methods**

The bacterial species identified from different preparation methods were determined using 16S rRNA gene sequencing, with the corresponding GenBank accession numbers (Table 3). Fresh samples primarily contained *Aeromonas caviae* and *Shewanella xiamenensis* (GenBank: AP019195.1, MG428945.1).

In Frozen samples, spore-forming bacteria such as *Bacillus pseudomycoides*, *Bacillus cereus*, and *Bacillus thuringiensis* (GenBank: MK999398.1,

CPO12691.1, CPO16194.1) were prevalent, alongside *Micrococcus endophyticus* and *Micrococcus luteus* (GenBank: MN474029.1, MH778045.1).

Heat-treated samples (Fried, Roasted, and Boiled) predominantly featured heat-resistant species, including *Bacillus subtilis*, *Bacillus amyloliquefaciens*, and *Bacillus velezensis* (GenBank: CP041691.1, CP033576.1, KU551225.1).

**Table 3: Bacterial Species Identified by Sample Type**

Name of Sample	Prediction	GenBank Accession
Fresh	<i>Aeromonas caviae</i> , <i>Shewanella xiamenensis</i>	AP019195.1, MG428945.1
Frozen	<i>Bacillus pseudomycoides</i> , <i>Bacillus cereus</i> , <i>Bacillus thuringiensis</i> , <i>Micrococcus endophyticus</i> , <i>Micrococcus luteus</i>	MK999398.1, CPO12691.1, CPO16194.1, MN474029.1, MH778045.1
Fried	<i>Bacillus subtilis</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus velezensis</i>	CP041691.1, CP033576.1, KU551225.1
Roasted	<i>Bacillus subtilis</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus cereus</i>	MR721058.1, MHH017383.1, KU218526.1
Boiled	<i>Bacillus subtilis</i> , <i>Bacillus amyloliquefaciens</i> , <i>Bacillus velezensis</i>	CP041691.1, CP033576.1, KU551225.1

## DISCUSSION

The bacteriological evaluation of *Pomadasys jubelini* highlights the significant effects of various processing methods on bacterial load, consistent with findings from previous studies on fish preservation. Fresh fish samples displayed the highest bacterial count ( $9.0 \times 10^8$  CFU/g), reflecting the natural microbial load typically found in raw fish due to environmental exposure, handling, and gut microbiota (Koralage *et al.*, 2023). In contrast, processing methods such as freezing, frying, boiling, and roasting resulted in a notable reduction in bacterial counts, with frying proving to be the most effective, yielding a minimal count of  $0.2 \times 10^8$  CFU/g.

This reduction in bacterial load from fresh to processed samples reinforces the effectiveness of cooking and preservation techniques, particularly frying and freezing, in inhibiting microbial growth (Jayasinghe *et al.*, 2022). Frying, in particular, diminishes bacterial contamination through high temperatures that disrupt bacterial cell walls and denature proteins, significantly reducing viability (Sani *et al.*, 2021). Similar studies have corroborated these findings, demonstrating that frying effectively lowers microbial loads in other fish species (Maharaj *et al.*, 2020).

Frozen samples exhibited a bacterial count of  $1.1 \times 10^8$  CFU/g, indicating that while freezing slows bacterial activity, it does not completely halt it. Freezing is known to inhibit growth by reducing water activity and altering cellular functions, yet certain psychrotrophic bacteria can survive this process (Zhu *et al.*, 2021). Thus, although freezing is beneficial for preservation, it may not be as effective as thermal methods in significantly reducing microbial populations.

The study identified several bacterial species, including *Bacillus*, *Micrococcus*, and *Shewanella*, which have been associated with spoilage and potential pathogenic effects in food products (Akpan *et al.*, 2020; Amaral *et al.*, 2019). The presence of *Bacillus cereus* is particularly concerning for food safety, as it is linked to foodborne illnesses, especially in fish and seafood (Jadhav *et al.*, 2022). These bacteria can survive mild heat treatments, meaning improper cooking or reheating may allow their spores to germinate, leading to contamination (Oudejans *et al.*, 2023). Additionally, *Shewanella xiamenensis* was isolated from fish and has been implicated in spoilage under refrigeration (Barbosa *et al.*, 2020). The isolation of *Micrococcus luteus* and *Micrococcus endophyticus* aligns with the findings of Akinyemi *et al.*, (2022), which noted that *Micrococcus* species are commonly found in aquatic environments and on fish surfaces. While they may

cause spoilage, they are generally not pathogenic to humans.

The application of molecular techniques, such as 16S rRNA sequencing, has proven essential for accurately identifying bacterial species, as shown by the GenBank accession data in this study. This methodology enhances bacterial identification and contributes to the growing understanding of the microbial ecology of seafood (Singh *et al.*, 2022). Furthermore, the use of molecular tools improves traceability and control of foodborne pathogens in fishery products, aligning with contemporary food safety protocols (Amagliani & Brandi, 2021).

## CONCLUSION

This study underscores the significance of effective fish processing methods for reducing microbial contamination in *Pomadasys jubelini*, with frying emerging as the most efficient technique for lowering bacterial loads. The identification of potential pathogens, such as *Bacillus cereus*, highlights the necessity for strict food safety protocols during the handling and processing of fish. While freezing, boiling, and roasting are beneficial, thermal processes like frying should be prioritized to minimize microbial risks.

The application of molecular techniques for bacterial identification enhances the accuracy of detecting potentially harmful microorganisms, thereby bolstering food safety initiatives. Future research should explore alternative preservation methods, such as vacuum packaging and the use of natural antimicrobials, to assess their effectiveness in reducing bacterial loads in fish products. Additionally, the increasing utilization of molecular tools for identifying and tracking foodborne pathogens represents a promising advancement in ensuring seafood safety.

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