Microbial Growth of *Staphylococcus Aureus* in the Gut of Indian Oil Sardine (*Sardinella Longiceps*): A Comparative Study of Preservation Techniques

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ABSTRACT

This study evaluated the effectiveness of three traditional preservation methods drying, salting, and smoking on the survival of *Staphylococcus aureus* in *Sardinella longiceps* (Indian oil sardine) in Estancia, Iloilo, a major artisanal fishing hub in the Philippines. Few studies have examined how these artisanal practices perform under real community conditions, despite their continued importance in local food security and fisheries livelihoods. A completely randomized design was employed, with microbial growth measured as colony-forming units (CFUs) on Mannitol Salt Agar. Results showed significant differences among treatments, with smoking producing the highest microbial reduction, followed by salting and drying. The findings provide evidence that smoking remains the most reliable traditional method for extending shelf life and reducing microbial risks, while highlighting the need to integrate traditional practices with modern food safety innovations to strengthen both public health and artisanal fishery sustainability.

Keywords: Sardinella longiceps, Staphylococcus aureus, drying, salting, smoking, artisanal fisheries, food safety

INTRODUCTION TO THE STUDY

This chapter introduces the study by discussing its background, rationale, objectives, significance, and scope, thereby establishing the foundation and direction of the research.

Fish is among the most perishable food commodities due to its high moisture content, enzymatic activity, and susceptibility to microbial contamination. Without preservation, spoilage leads not only to nutritional loss but also to food safety risks and economic waste. For fishing-dependent communities, these issues extend beyond biology and directly influence livelihoods, health, and social well-being (Duan et al., 2021; Mahmud et al., 2018).

The Indian oil sardine (*Sardinella longiceps*, locally known as “Tuloy”) is an abundant and affordable food source in the Philippines, particularly in coastal communities such as Estancia, Iloilo. It contributes significantly to the daily diet of low-income households while also sustaining local fish vendors and processors. However, its high perishability makes safe preservation crucial. Inadequate handling not only increases the risk of foodborne illness but also causes post-harvest losses, reducing household income and threatening food security.

One major foodborne pathogen of concern is *Staphylococcus aureus*. This halotolerant bacterium can survive in salted and dried fish, form biofilms, and produce heat-stable enterotoxins that remain active even after cooking (Onyenweaku et al., 2024; Silva et al., 2023). Its persistence in artisanal fish products poses public health risks, particularly in communities with limited access to cold storage and sanitary facilities. "Traditional fish preservation techniques, such as drying, salting, and smoking, remain central to artisanal fisheries but require improvements to ensure food safety and consumer protection (Li et al., 2024)."

Although modern preservation technologies such as freezing, canning, and high-pressure processing are highly effective, they remain inaccessible to small-scale fishers due to cost. As a result, artisanal preservation methods drying, salting, and smoking continue to dominate in coastal settings. These practices are not merely technical solutions but also cultural traditions and livelihood strategies passed down through generations.

This study investigates the effectiveness of three traditional preservation techniques on the survival of *S. aureus* in *S. longiceps*. By combining microbiological evidence with social context, the research addresses two dimensions: (1) the biological effectiveness of preservation against microbial contamination, and (2) the social and economic implications of preservation practices for artisanal fishers and consumers. This dual perspective highlights how food safety directly intersects with community resilience, public health, and sustainable livelihoods.

Background and Rationale:

Fish and fishery products are among the most perishable food commodities, requiring effective preservation to maintain quality, extend shelf life, and ensure safety. Preservation slows spoilage by limiting microbial activity and biochemical changes that compromise product quality. Traditional methods such as drying, salting, pickling, and smoking have long been used, while modern techniques such as freezing, canning, and high-pressure processing are now widely adopted (Mahmud et al., 2018; Tavares et al., 2021).

For the Indian oil sardine (*Sardinella longiceps*, locally known as Tuloy), preservation is essential to prevent rapid deterioration and safeguard its nutritional value. As a widely consumed low-cost protein source, sardines are central to food security in the Philippines. Effective preservation reduces foodborne illness risks, minimizes post-harvest losses, and ensures safer consumption. Studies have highlighted the potential of natural bio-preservatives, such as extracts from *Sargassum wightii* and *Coleus aromaticus*, which significantly extend the shelf life of sardine fillets by inhibiting spoilage microorganisms during refrigerated storage (Athira et al., 2020).

In coastal communities like Estancia, Iloilo, fish preservation has direct implications for both public health and livelihood. While fish contributes high-quality protein, vitamins, and minerals, inadequate preservation increases the risk of microbial contamination, undermining both consumer safety and the economic value of fishery products (Mahmud et al., 2018).

Advances in physical methods, including refrigeration, freezing, and high-pressure processing, effectively inactivate spoilage and pathogenic organisms without compromising sensory or nutritional properties (Tavares et al., 2021). Similarly, chemical preservatives such as EDTA, TBHQ, and ascorbic acid reduce oxidative and microbial spoilage, prolonging shelf life (Ghaly, 2020). Emerging technologies, such as pulsed electric fields, pulsed light, ultrasound, electrolyzed water, and non-thermal plasma, further extend shelf life while maintaining nutritional and sensory integrity (Speranza et al., 2021; Rathod et al., 2022).

Natural antimicrobials, including lysozyme, lactoferrin, chitosan, and essential oils, have proven effective against spoilage organisms and resistant bacterial strains. Microbial-based preservatives such as bacteriocins, reuterin, and pediocin also demonstrate strong inhibitory effects (Teshome et al., 2022). These approaches highlight innovative options for ensuring safety and quality. However, their adoption in artisanal fisheries remains limited due to cost and accessibility, reinforcing the continued reliance on traditional methods.

This study addresses that gap by comparing drying, salting, and smoking under local artisanal conditions, with a focus on the survival of *Staphylococcus aureus*, a halotolerant foodborne pathogen of public health concern.

Conceptual Framework

This study examined the effectiveness of three traditional preservation methods drying, salting, and smoking on the survival of Staphylococcus aureus in *Sardinella longiceps.*

This study investigated the effectiveness of three traditional preservation methods drying, salting, and smoking—on the survival of Staphylococcus aureus in *Sardinella longiceps.* The preservation techniques

served as the independent variables, while the microbial growth of S. aureus, measured in terms of colony-forming units (CFU) on Mannitol Salt Agar, represented the dependent variable. The indicators of effectiveness were assessed through reductions in CFU counts, extension of shelf life, and improvements in food safety.

The mechanisms underlying these preservation methods vary considerably. Drying reduces the moisture content of fish, thereby lowering water activity essential for microbial metabolism. Salting induces osmotic stress that dehydrates microbial cells, though halotolerant species such as S. aureus may continue to survive under such conditions. Smoking, by contrast, exerts a combined effect through heat application, moisture reduction, and the deposition of antimicrobial compounds present in smoke, including phenols and organic acids. Together, these mechanisms provide differing levels of inhibition, highlighting the need for comparative evaluation under artisanal conditions.

I. Dependent Variable: Microbial growth of *S. aureus*, measured as colony-forming units (CFU) on Mannitol Salt Agar
II. Indicators of Effectiveness: Reduction in CFU counts, shelf-life extension, and improved food safety

Each method acts through distinct mechanisms:

1. Drying lowers moisture content, reducing water activity needed for microbial metabolism.
2. Salting creates osmotic stress that dehydrates cells, though halotolerant species like *S. aureus* may persist.
3. Smoking combines heat, moisture reduction, and antimicrobial compounds in smoke (phenols, organic acids), producing stronger inhibitory effects.

While this study focuses on the microbial inhibition achieved through drying, salting, and smoking, emerging biopreservation strategies such as the use of lactic acid bacteria are increasingly explored as complementary methods (Cortés-Sánchez et al., 2024)."

Social Dimension

While these mechanisms are microbiological, their outcomes affect community health, household food security, and fishing livelihoods. Ineffective preservation increases the risk of foodborne illness, reduces consumer trust, and results in higher post-harvest losses. Conversely, effective methods enhance food safety, extend the marketability of fish, and provide economic resilience for small-scale fishers. Thus, the framework links preservation methods → microbial safety → social and economic outcomes.

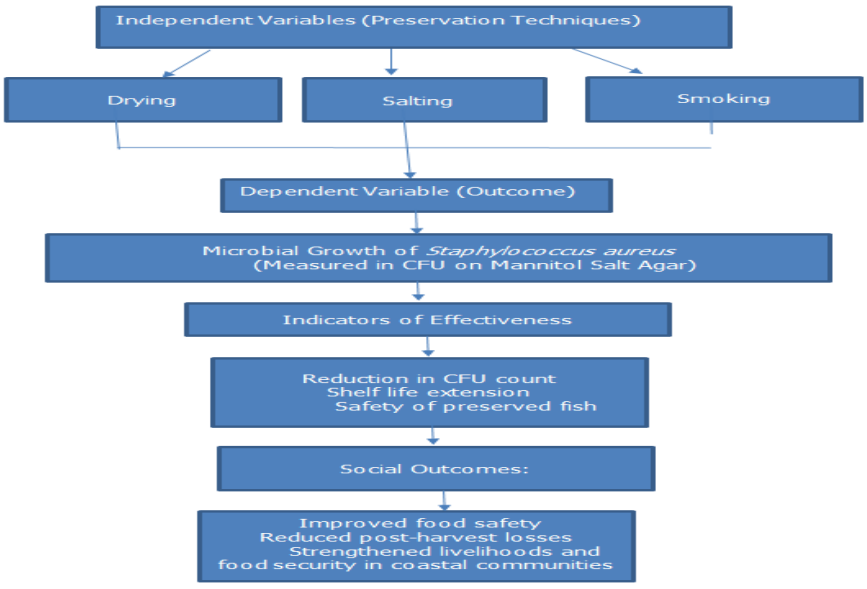

Figure 1. Shows the effects of drying, salting, and smoking on the microbial growth of Staphylococcus aureus measured on Mannitol Salt Agar (in CFU) and social outcomes.

Objectives of the Study

This study aimed to evaluate the effectiveness of three traditional preservation techniques drying, salting, and smoking on the microbial growth of *Staphylococcus aureus* in *Sardinella longiceps*. Specifically, it sought to:

1. Quantify the colony-forming units (CFU) of *S. aureus* in sardines subjected to drying, salting, and smoking.
2. Compare the preservation methods to determine significant differences in their effectiveness.
3. Relate the outcomes of microbial safety to social implications, including food security, public health, and artisanal livelihoods in coastal communities.

Significance of the Study

The findings of this study are significant on scientific, practical, and societal levels.

Scientific significance. This research contributes to the growing literature on the microbial safety of traditionally preserved fish, focusing on Staphylococcus aureus, a halotolerant pathogen capable of surviving salting and drying while producing heat-stable toxins (Chieffi et al., 2023; Onyenweaku et al., 2024). By directly comparing drying, salting, and smoking of Sardinella longiceps under local artisanal conditions, the study advances knowledge of microbial dynamics in traditional fish preservation, a field where research remains limited in the Philippines and other tropical regions (Mahmud et al., 2018; Duan et al., 2021).

Practical significance. The study provides evidence-based insights to help artisanal processors, vendors, and consumers adopt preservation methods that are both cost-effective and microbiologically safer. These findings are particularly valuable for communities with limited access to modern preservation infrastructure, where traditional methods remain the primary means of extending fish shelf life (Oliveira et al., 2021; Ahmed et al., 2021). Results may also guide the development of locally adapted food safety guidelines and training programs to improve post-harvest handling (Silva et al., 2023).

Societal significance. This research addresses food security, nutrition, and public health in fishing-dependent communities. Spoiled fish contributes not only to economic losses but also to heightened risks of foodborne illness, especially in low-resource settings (Nizio et al., 2023; Romano et al., 2024). Identifying preservation methods that effectively limit S. aureus growth supports public health protection, reduces food waste, and strengthens the economic resilience of small-scale fishers (Rathod et al., 2022; Ghaly, 2020).

In sum, this study integrates scientific inquiry with community application by situating artisanal fish preservation within contemporary food safety frameworks. Its outcomes are expected to benefit both fisheries microbiology scholarship and the daily practices of coastal communities that rely on sardines as a staple food source.

Scope and Delimitations of the Study

This study focused on evaluating the effectiveness of three traditional preservation methods, drying, salting, and smoking, on the microbial growth of *Staphylococcus aureus* in the Indian oil sardine (*Sardinella longiceps*). The experiment was conducted under artisanal conditions in Estancia, Iloilo, reflecting practices commonly used in local coastal communities. Microbiological analysis centered on quantifying colony-forming units (CFU) of *S. aureus* using Mannitol Salt Agar to determine the relative effectiveness of each preservation method.

The study was delimited to:

1. Preservation methods – Only drying, salting, and smoking were tested. Other traditional or modern preservation techniques (e.g., pickling, freezing, canning) were not included.
2. Target organism – Analysis was limited to *S. aureus* as a representative halotolerant foodborne pathogen. Other spoilage organisms and pathogens were not covered.

3. Study area and conditions –Experiments were conducted using locally available resources and artisanal practices in Estancia, Iloilo. Industrial-scale or laboratory-optimized preservation techniques were excluded.
4. Sample type – The study used fresh *S. longiceps* sourced from local fishers. Results may not directly apply to other fish species or to sardines processed under commercial or industrial settings.

By defining these boundaries, the study provides context-specific evidence on the microbial safety of artisanal fish preservation methods while acknowledging that findings cannot be generalized to all preservation practices or seafood products.

Definition of Terms

To have a better understanding of the study, the following terms were conceptually and operationally defined:

1. Colony-Forming Unit (CFU): The number of viable *Staphylococcus aureus* colonies observed on Mannitol Salt Agar plates in this study, serving as the quantitative indicator of microbial growth.
2. Drying: A preservation method applied in this study by air- or sun-drying *Sardinella longiceps* to reduce moisture content and water activity, tested for its effectiveness in lowering *S. aureus* counts.
3. Estancia, Iloilo: The artisanal fishing community in the Philippines where this study was conducted, recognized for its reliance on traditional fish preservation methods such as drying, salting, and smoking.
4. Halotolerant Microorganisms: Bacteria, including *Staphylococcus aureus*, capable of surviving and multiplying in high-salt environments, which explains why salting alone may not completely inhibit growth in fish products.
5. Mannitol Salt Agar (MSA): The selective culture medium used in this research to isolate and enumerate *S. aureus* from fish samples after preservation treatments.
6. Microbial Growth: Refers to the increase in *S. aureus* colonies in preserved sardines, measured through CFU counts in this study.
7. Osmotic Stress: The process induced by salting in this study, where water is drawn out of microbial cells, inhibiting growth; assessed to determine the limits of salt preservation against *S. aureus*.
8. Preservation Methods: The three traditional fish preservation techniques drying, salting, and smokingapplied to *Sardinella longiceps* in this research to evaluate their impact on *S. aureus* survival.
9. Salting: The application of salt to sardine samples in this study to reduce microbial growth by creating osmotic stress, tested at concentrations commonly used in artisanal fisheries.
10. Shelf Life: The duration during which preserved sardines remained safe and consumable in this study, inferred from microbial counts and reduction of *S. aureus*.
11. Smoking: A preservation method applied in this research where sardine samples were exposed to heat and smoke, combining moisture reduction and antimicrobial compounds to evaluate effectiveness against *S. aureus*.
12. Staphylococcus aureus: The halotolerant, foodborne bacterium examined in this study as the test organism to assess microbial risks associated with traditional fish preservation.
13. Traditional Preservation: The artisanal fish-processing practices (drying, salting, smoking) still commonly used in Estancia, Iloilo, which were experimentally tested in this study for microbial safety.

REVIEW OF RELATED LITERATURE

Fish preservation has long relied on traditional methods such as drying, salting, and smoking, which slow microbial growth but vary in effectiveness (Ahmed et al., 2021; Dhakal, 2022; Oliveira et al., 2021). While these methods extend shelf life, halotolerant organisms such as *Staphylococcus aureus* often survive and may produce toxins that threaten consumer health (Chieffi et al., 2023; Silva et al., 2023). This poses serious concerns not only for microbiology but also for public health and food security in artisanal communities.

Recent studies have explored natural preservatives and innovative technologies. Essential oils, bacteriocins, edible coatings, ultrasound, and high-pressure processing show promise for suppressing spoilage organisms while maintaining sensory quality (Alfonzo et al., 2017; Kontominas et al., 2021; Rizzo et al., 2024). Modified

atmosphere packaging and cold storage also improve fish safety, but such methods remain costly and largely inaccessible to small-scale fishers (MDPI, 2022; Ramaswamy & Chen, 2022).

Despite these advances, microbial contamination in artisanal fisheries persists. Studies confirm that salting reduces microbial loads but cannot fully prevent pathogen persistence (Aoua et al., 2024; Onyenweaku et al., 2024). This reality has economic and social consequences: unsafe products erode consumer trust, increase post-harvest losses, and reduce the income of coastal households that rely on fishing as their primary livelihood (Nizio et al., 2023; Romano et al., 2024).

Research Gap

Although preservation strategies have been widely studied, few investigations directly examine how traditional artisanal methods perform under community-based conditions in the Philippines. Moreover, limited attention has been given to how preservation intersects with socio-economic resilience, particularly for vulnerable fishing communities with limited access to modern infrastructure.

Contribution of the Present Study

This study addresses these gaps by comparing drying, salting, and smoking under real artisanal conditions, focusing on the survival of *S. aureus* in *Sardinella longiceps*. Beyond microbiological safety, it emphasizes the social significance of preservation: ensuring safer diets, reducing waste, and strengthening livelihoods. By linking scientific evidence to community practice, the study situates fish preservation not only within food microbiology but also within social science perspectives on food security, health, and sustainability.

RESEARCH DESIGN & METHODOLOGY

This chapter presents the procedures used to evaluate the effectiveness of drying, salting, and smoking in reducing the growth of *Staphylococcus aureus* in *Sardinella longiceps*, including the research design, study area, materials, and data analysis.This study used a completely randomized experimental design to evaluate the effectiveness of three traditional preservation methods drying, salting, and smoking on the growth of *Staphylococcus aureus* in *Sardinella longiceps*. Fresh sardines were purchased from the Estancia Public Market and assigned to treatment groups.

Microbiological analysis involved serial dilution, spread plating on Mannitol Salt Agar, and enumeration of colony-forming units (CFUs). Identity confirmation of *S. aureus* was conducted using the catalase test. Data were analyzed using descriptive statistics and one-way ANOVA, followed by Tukey's HSD test to compare treatments.

All procedures adhered to institutional biosafety standards.

Figure 2. Map of the Philippines showing the location of the study area in Estancia, Iloilo Philippines.

Note: Figure 2 presents the geographical location of the study area in Estancia, Iloilo, Philippines. The map highlights its position within the Visayas region, helping contextualize the study site.

The map shown in Figure 2 provides a visual representation of the study area located in Estancia, a coastal municipality in the province of Iloilo, Philippines.

Estancia is part of the Visayas region, situated in the central part of the country.

The map helps readers identify the precise location of the study site in relation to other major islands and provinces in the Philippines.

This geographic context is essential for understanding environmental and regional factors that may influence the outcomes of the research. Estancia's coastal location is particularly relevant for studies involving marine resources, fisheries, or environmental conditions affected by human and natural activities in coastal communities.

Collection of Samples

Fish samples of Indian Oil Sardine *(Sardinella longiceps*) were collected from the coastal area of Estancia, Iloilo. These samples were purchased directly from the local market in Estancia, ensuring they were freshly caught from the surrounding coastal waters. This approach guaranteed high-quality specimens for the study.

Figure 3. Illustrates the purchase of freshly caught Indian Oil Sardine (*Sardinella longiceps*) from the local market in Estancia, Iloilo

Note: Figure 3 shows the purchase of freshly caught Indian Oil Sardine *(Sardinella longiceps)* at the local market in Estancia, Iloilo. The image highlights the availability and handling of sardines, which are commonly sourced by local fishers and sold directly to consumers in the region.

Research Design

This study employed a completely randomized design to investigate microbial growth in Indian oil sardine (*Sardinella longiceps*) and evaluate the effectiveness of three traditional preservation techniques: drying, salting, and smoking. Fresh sardines of uniform size and weight were sourced from a local fish market and randomly assigned to treatment groups corresponding to each preservation method. Drying was carried out through sun exposure to reduce moisture content; salting applied varying concentrations of sodium chloride to create osmotic stress; and smoking utilized traditional hot smoking to combine heat and antimicrobial smoke compounds. The preserved samples were stored under controlled room conditions for one week before gut extractions were conducted.

Microbial load was assessed through colony-forming unit (CFU) analysis, while biochemical confirmation of *Staphylococcus aureus* was performed using the Catalase test. Statistical analysis employed a one-way Analysis of Variance (ANOVA) to compare the prevalence of *S. aureus* across treatments, followed by Tukey's Honestly Significant Difference (HSD) post hoc test to identify specific differences among preservation methods.

The findings provide insights into the microbial stability of *S. longiceps* under artisanal preservation practices. This research holds practical value for small-scale fish processors, traders, and consumers who rely on traditional methods, while also contributing to the broader scientific understanding of food safety in coastal communities.

Research Locale

This study was conducted in Estancia, a second-class municipality in Iloilo Province, Philippines, located about 135 kilometers from Iloilo City. Known as the "Alaska of the Philippines" for its abundant marine resources, Estancia plays a vital role in the regional fishing industry. Its modern fish port, one of the most developed in northern Visayas, serves as a central hub for landing and processing sardines and other commercially important species from the nearby waters of Carles.

Fishing in Estancia primarily involves purse seining, trawling, and gill netting—methods suited to the shallow waters of the Visayan Sea. The area is part of the Sulu-Sulawesi Triangle, a region recognized for its rich marine biodiversity. However, concerns over pollution and overfishing have raised sustainability issues that heighten the need for effective preservation practices.

The fishing industry is deeply embedded in Estancia's community, with an estimated 60% of households dependent on it for livelihood. This strong reliance on fisheries underscores the importance of ensuring that catch, particularly sardines, is preserved safely and efficiently. These conditions make Estancia a highly relevant setting for studying microbial growth in *Sardinella longiceps* and for comparing the effectiveness of traditional preservation techniques such as drying, salting, and smoking.

Numerous dealers engaged in wholesale and retail fish trade further emphasize Estancia's economic importance as a fisheries hub. At the same time, growing awareness of environmental pressures on marine resources has prompted local initiatives such as the deployment of artificial reefs to restore fish populations.

Conducting research on microbial growth in sardines within this setting provides valuable insights into effective preservation strategies. Beyond its scientific contribution, the study has the potential to strengthen food security and promote sustainable fishing practices. In doing so, it supports Estancia's role as a vital commercial fishing center while contributing to the long-term protection of its marine biodiversity.

Quantitative Approach

This study employed an experimental quantitative design to evaluate the effects of traditional preservation techniques, drying, salting, and smoking on the microbial growth of *Staphylococcus aureus* in the gut of Indian oil sardines (*Sardinella longiceps*). The experiment was carried out under controlled laboratory conditions to ensure consistency across treatments.

Microbial load was assessed by enumerating colony-forming units (CFUs) of *S. aureus* cultured on Mannitol Salt Agar (MSA). A catalase test was performed to confirm the identity of the microorganism.

Statistical analysis was conducted using one-way Analysis of Variance (ANOVA) to compare microbial growth across the three preservation methods. ANOVA was chosen as it is appropriate for comparing the means of three or more independent groups when the data are approximately normally distributed and the variances are homogeneous, conditions that were satisfied by the dataset. Following the ANOVA, a post hoc analysis using Tukey's Honestly Significant Difference (HSD) test was performed to identify pairwise differences and determine which preservation methods significantly differed in their effectiveness against *S.*

aureus.

This approach ensured rigorous measurement and reliable comparison of preservation techniques, directly addressing the study's objectives of quantifying and contrasting the effectiveness of drying, salting, and smoking.

Data Gathering Procedure

Approval to conduct this research was obtained from the Research Adviser, the Dean of the College of Arts and Sciences, and the University President of Northern Iloilo State University. The study followed institutional guidelines for scientific research and adhered to ethical standards in microbiological investigation.

Fish samples used in this study were sourced from local markets in Estancia, Iloilo, and handled with care to ensure hygienic and responsible use of marine resources. No live vertebrate animals or human participants were involved, and thus the study did not require animal welfare or human subject clearance.

All microbiological procedures were performed in accordance with standard biosafety protocols to minimize risks to researchers and the environment. Cultures of *Staphylococcus aureus* were handled in a controlled laboratory setting, and all contaminated materials were properly sterilized and disposed of following laboratory safety procedures.

By observing these ethical and biosafety measures, the study ensured compliance with institutional requirements while safeguarding public health, laboratory personnel, and the environment.

Data Analysis

The researchers employed both descriptive and inferential statistical analyses to examine the microbial growth of *Staphylococcus aureus* in the gut of Indian oil sardine (*Sardinella longiceps*) subjected to drying, salting, and smoking. Descriptive statistics were used to summarize and present the colony-forming unit (CFU) counts obtained for each preservation method.

Inferential analysis was then conducted to evaluate whether differences in microbial growth among the treatment groups were statistically significant. A one-way Analysis of Variance (ANOVA) was performed to compare the mean CFU counts across the three preservation techniques. Where significant variation was detected, Tukey's Honestly Significant Difference (HSD) post hoc test was applied to identify specific group differences.

These analytical procedures provided a reliable basis for assessing the comparative effectiveness of traditional preservation methods in controlling *S. aureus* contamination in sardines.

METHODS

Fish Acquisition and Processing

Fresh Indian oil sardines (*Sardinella longiceps*) were purchased from the Estancia Public Market and divided into three groups of ten fish each. The groups were assigned to preservation treatments: drying, salting, and smoking. This procedure allowed systematic comparison of microbial growth under different preservation methods. By observing the samples, the researchers assessed which method delayed the growth of *Staphylococcus aureus* most effectively. To avoid ethical concerns, fish were sourced from the market rather than live capture, ensuring no animal welfare issues were involved.

Preparation of Agar Media

Mannitol Salt Agar (MSA) was prepared according to manufacturer specifications by dissolving the medium in distilled water, sterilizing it by autoclaving at 121°C and 15 psi for 30 minutes, and cooling to 45–50°C before pouring into sterile Petri dishes under aseptic conditions. A total of 36 plates were prepared to accommodate

all treatments and replicates.

Serial Dilution Procedure

To quantify microbial load, a 10-fold serial dilution was performed for each preservation method. Six sterile test tubes were prepared with 10 mL of distilled water each. One milliliter of homogenized fish gut sample was transferred into the first tube (10^{-1}) and mixed thoroughly. This step was repeated sequentially up to 10^{-6}, with sterile pipette tips used at each transfer to prevent cross-contamination. Identical procedures were carried out for the drying, salting, and smoking treatments.

Spread Plating and Incubation

Aliquots from the dilution series were plated onto MSA using a sterile disposable spreader. Each treatment was plated in nine replicates to ensure accuracy and reproducibility. Plates were incubated in an inverted position at 37°C for 24 hours, providing optimal conditions for *S. aureus* colony development.

Determination of Colony-Forming Units (CFU)

Following incubation, visible colonies of *S. aureus* were counted and recorded as CFU per gram of sample. This provided a comparative assessment of microbial load across the three preservation techniques and allowed evaluation of their effectiveness in reducing bacterial growth.

Catalase Test

To confirm the identity of *S. aureus*, a catalase test was performed on representative isolates. The test involved applying hydrogen peroxide to bacterial cultures to detect the presence of the catalase enzyme, characteristic of *S. aureus* (Garcia & Santos, 2021).

Presentation, Analysis, and Interpretation of Data

This chapter presents the results of the study on the effectiveness of drying, salting, and smoking in preserving Indian oil sardine (Sardinella longiceps, locally known as “Tuloy”) in Estancia, Iloilo. The findings are organized into three sections: (1) bacterial load (colony-forming units, CFU), (2) results of the one-way ANOVA, and (3) post hoc comparisons using Tukey’s Honestly Significant Difference (HSD) test. Each section includes analysis and interpretation in relation to the study objectives and relevant literature.

Colony-Forming Units (CFU) of Staphylococcus aureus Under Different Preservation Techniques

Table 1 summarizes the CFU counts of S. aureus in sardines preserved by drying, salting, and smoking, compared with a control group. The table includes mean CFU values, standard deviations (SD), and replicates (N), based on the optimal dilution factor of 10^{-4}.

Drying recorded the highest bacterial load ($\bar{x}$ = 50,000,000.00; SD = 5,567,764.36), indicating that it was the least effective method in suppressing S. aureus. This suggests that drying alone may still provide favorable conditions for microbial survival.

Salting produced a markedly lower mean CFU ($\bar{x}$ = 5,366,666.67; SD = 7,480,864.48), reflecting a substantial inhibitory effect. However, the large SD highlights variability in outcomes, which may be attributed to differences in salt penetration or environmental conditions.

Smoking also reduced microbial growth ($\bar{x}$ = 6,500,000.00; SD = 7,365,459.93), showing a similar inhibitory effect to salting, though variability among replicates was also noted.

Interestingly, the control group had the lowest CFU ($\bar{x}$ = 3,000,000.00; SD = 0.00). While counterintuitive, this may suggest that untreated samples reflected only the baseline microbial load at the start of storage, whereas preserved samples provided conditions for differential microbial responses over time.

Overall, salting and smoking were more effective than drying in limiting S. aureus. This aligns with the findings of Barcenilla et al. (2022), who reported that both methods inhibit bacterial growth by creating unfavorable osmotic and chemical conditions. Likewise, Alp and Bulantekin (2021) observed that although drying reduces moisture, its success in controlling microbial proliferation depends heavily on environmental factors, which can allow halotolerant organisms such as S. aureus to persist. are not optimal.

Table 1. The colony forming units (CFU) of Staphylococcus aureus under different preservation techniques, including drying, salting, and smoking

Technique	**Mean**	**SD**	**N**
Control	3.00×10^6	0.0×10^0	3
Drying	5.00×10^7	5.57×10^6	3
Salting	5.37×10^6	7.48×10^6	3
Smoking	6.50×10^6	7.37×10^6	3

Note: Table 1 shows the colony forming units (CFU) of Staphylococcus aureus under different preservation techniques. Smoking resulted in the highest CFU, followed by salting and drying. The control group had the lowest CFU. This suggests that preservation methods can significantly influence bacterial growth. Data are based on three replicates.

Significant Differences in the CFU of *Staphylococcus aureus* Across Preservation Techniques

A one-way Analysis of Variance (ANOVA) was performed to compare the CFU counts of *Staphylococcus aureus* in sardines preserved by drying, salting, and smoking, alongside a control group.

The results revealed a statistically significant difference among treatments, **$F(3, 8) = 38.56$, $p < .001$**. This indicates that the preservation method exerted a significant effect on microbial growth. The between-group variance ($SS = 4.084 \times 10^{15}$, $MS = 1.361 \times 10^{15}$) was substantially greater than the within-group variance ($SS = 2.824 \times 10^{14}$, $MS = 3.530 \times 10^{13}$), confirming that not all techniques had the same inhibitory effect on *S. aureus*.

These findings demonstrate that at least one of the preservation techniques—drying, salting, or smoking—significantly differed in its ability to reduce bacterial proliferation, justifying the use of Tukey's HSD post hoc test for pairwise comparisons.

The significant p-value (< .001) suggests strong evidence against the null hypothesis, confirming that Staphylococcus aureus colony counts are not equal across all preservation treatments. Further post hoc analysis (Tukey HSD) would be appropriate to identify which specific techniques differ from each other.

Table 2. The significant difference in the colony forming units (CFU) of Staphylococcus aureus under different preservation techniques, including drying, salting, and smoking

	Sum of Squares	**df**	**Mean Square**	**F**	**Sig**	**Interpretation**
Between Groups	4.084E+15	3	1.361E+15	38.557	<.001	Significant
Within Groups	2.824+4	8	3.530E+13			
Total	4.366E+15	11				

Note: Table 2 presents ANOVA results showing a statistically significant difference ($p < .001$) in CFU of Staphylococcus aureus among preservation techniques. With $F = 38.557$ and a significance level below 0.05, the findings confirm that drying, salting, and smoking significantly affect bacterial growth compared to the control group.

Multiple Comparisons of *Staphylococcus aureus* CFU Using Tukey's HSD Test

Tukey's HSD test showed statistically significant differences between the control group and all preservation methods: drying ($p = .014$), salting ($p = .004$), and smoking ($p = .006$). These results confirm that preservation treatments altered microbial behavior compared with untreated samples. Notably, the control group exhibited a lower baseline CFU mean, while the treated groups reflected microbial shifts influenced by preservation processes.

Further pairwise comparisons revealed significant differences between drying and salting ($p < .001$) and between drying and smoking ($p < .001$). Drying was consistently less effective in reducing *S. aureus* growth, likely due to residual moisture, variable environmental conditions during dehydration, and the absence of antimicrobial compounds present in salting and smoking.

By contrast, salting and smoking did not significantly differ ($p = .995$). Both methods created comparably hostile environments for *S. aureus*: salting through osmotic stress and dehydration of bacterial cells, and smoking through the combined effects of heat, moisture reduction, and antimicrobial compounds such as phenols and organic acids.

Together, these results confirm that while all three methods influence bacterial dynamics, salting and smoking provide more reliable inhibition of *S. aureus* than drying.

Table 3.Tukey HSD Test for Multiple Comparisons of Staphylococcus aureus Colony Forming Units (CFU)

	Techniques	**Sig.**	**Interpretation**
Control	Drying	.014	Significant
	Salting	.004	Significant
	Smoking	.006	Significant
Drying	Salting	<.001	Significant
	Smoking	<.001	Significant
Salting	Smoking	.995	Not Significant

Note: Table 3 shows Tukey HSD results indicating significant differences in CFU between most preservation methods. Drying and smoking significantly differ from control and salting. However, salting and smoking do not significantly differ ($p = .995$), suggesting similar effects on Staphylococcus aureus growth. All other pairwise comparisons were statistically significant.

These findings are supported by previous studies. Feng et al. (2022) reported that preservation techniques significantly influence the survival of Staphylococcus aureus, with drying being less effective than salting and smoking. They observed that the variability in drying conditions, including humidity and temperature, often results in inconsistent reductions in bacterial load. In contrast, salting and smoking provide more consistent and potent antimicrobial effects. Similarly, Fraqueza et al. (2020) found that salting and smoking contribute to the creation of antimicrobial environments that inhibit bacterial growth, while the efficacy of drying depends heavily on environmental conditions and the drying process's efficiency.

This finding is consistent with earlier observations that drying reduces water activity but may also lead to physicochemical and nutritional changes that allow microbial persistence (Fitri et al., 2022).

SUMMARY, FINDINGS, CONCLUSION, AND IMPLICATIONS

The results demonstrated that smoking was the most effective method in reducing *S. aureus* counts, followed by salting and drying. This agrees with studies highlighting smoking's combined effects of heat, reduced moisture, and antimicrobial compounds (Ahmed et al., 2021; Jackowska-Tracz et al., 2023). Recent findings also note smoking's consistent microbial safety benefits, though concerns remain over polycyclic aromatic hydrocarbons (Rizzo et al., 2024).

Salting produced moderate inhibition, consistent with Dhakal (2022) and Onyenweaku et al. (2024), who reported persistence of halotolerant *S. aureus* in salted fish. This reinforces that while salting can extend shelf life, it cannot guarantee complete pathogen control.

Drying was least effective, supporting Oliveira et al. (2021) and Fitri et al. (2022), who showed that drying reduces water activity but may allow microbial survival due to physicochemical changes in fish tissue. This indicates that drying should be combined with other preservation strategies for improved safety.

By situating these results within existing literature, this study extends current knowledge by demonstrating how traditional preservation methods perform under real artisanal conditions in Estancia, Iloilo. Beyond validating smoking as the most reliable traditional practice, the findings highlight opportunities to integrate natural antimicrobials (Pardo & López, 2024) and biopreservation approaches (Cortés-Sánchez et al., 2024) with artisanal techniques. Such integration reflects Li et al. (2024), who emphasize balancing cultural traditions with modern innovations for both consumer protection and community sustainability.

Findings

Significant differences were observed in bacterial load among preservation methods. Drying reduced microbial growth but was the least effective. Salting achieved the greatest inhibition of *S. aureus*, followed closely by smoking, with both showing significantly lower CFU counts than drying. ANOVA confirmed these differences ($p < 0.05$), and Tukey's test revealed salting and smoking to be comparable in effectiveness, both superior to drying.

Conclusion

This study demonstrated that smoking was the most effective traditional preservation method for reducing *Staphylococcus aureus* in *Sardinella longiceps*, followed by salting and drying. These findings validate smoking as the most reliable technique for artisanal fisheries in Estancia, Iloilo, while highlighting the limited effectiveness of drying and the moderate inhibitory effect of salting.

Beyond confirming the effectiveness of traditional methods, this study underscores the need for refinement and integration with modern food safety approaches. Recent literature emphasizes that combining traditional preservation with bio preservation strategies, such as lactic acid bacteria, or with natural antimicrobials, can further enhance food safety and sustainability (Cortés-Sánchez et al., 2024; Li et al., 2024). Therefore, future initiatives should focus on standardizing artisanal practices while adopting innovative methods that safeguard consumer health, prolong shelf life, and support the economic sustainability of fishing communities.

Implications

Scientific: Provides novel evidence on microbial control in artisanal fish preservation, particularly against *S. aureus*, a halotolerant and toxin-producing pathogen.

Practical: Guides processors and vendors in choosing cost-effective, safer preservation methods, with salting and smoking emerging as reliable options.

Societal: Supports food security and economic resilience in coastal communities by reducing post-harvest losses and minimizing foodborne health risks

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APPENDIX B

Narrative & Photo Documentation

Figure 1. Drying

Figure 2. Smoking

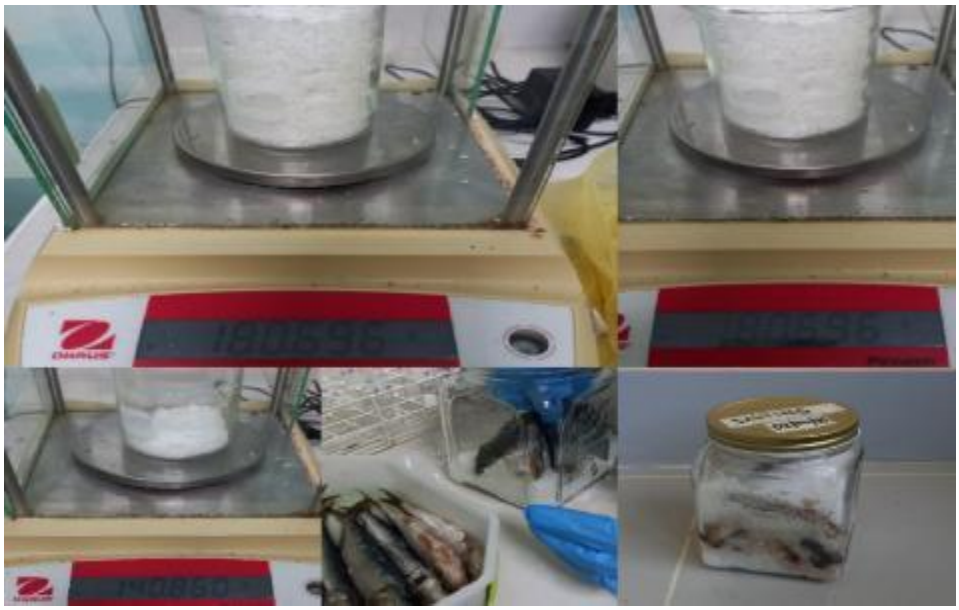

Figure 3. Salting

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Figure 4. Preserved Indian Oil Sardines Using Drying, Smoking, and Salting Methods

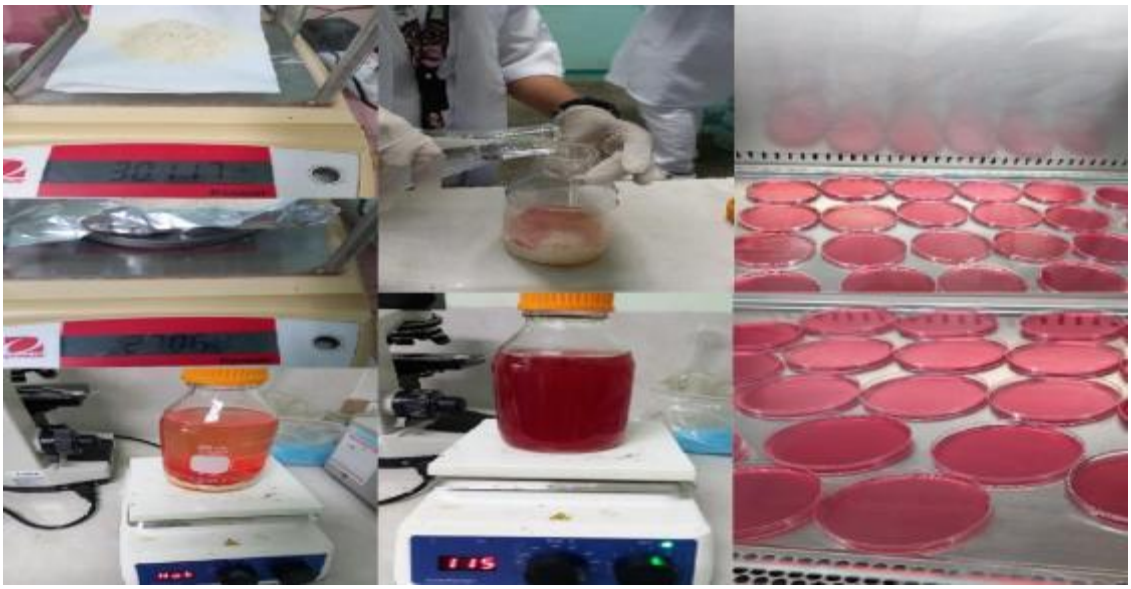

Figure 5. Preparation and Pouring of Mannitol Salt Agar (MSA) into Petri Dishes

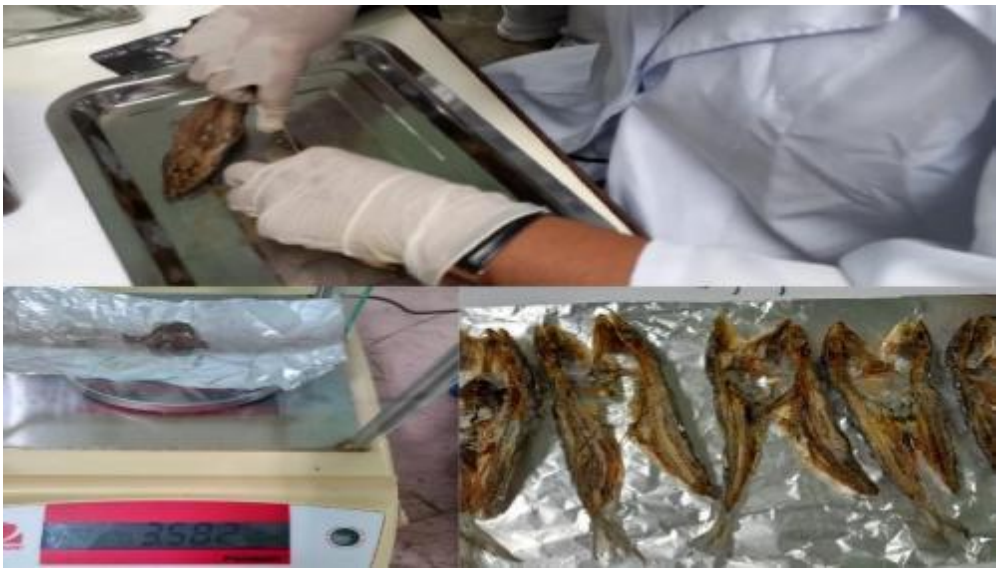

Figure 6. Gut of the Indian Oil Sardine (*Sardinella longiceps*) was extracted prior to preservation by drying,

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salting, and smoking (Drying)

Salting 3. Smoking

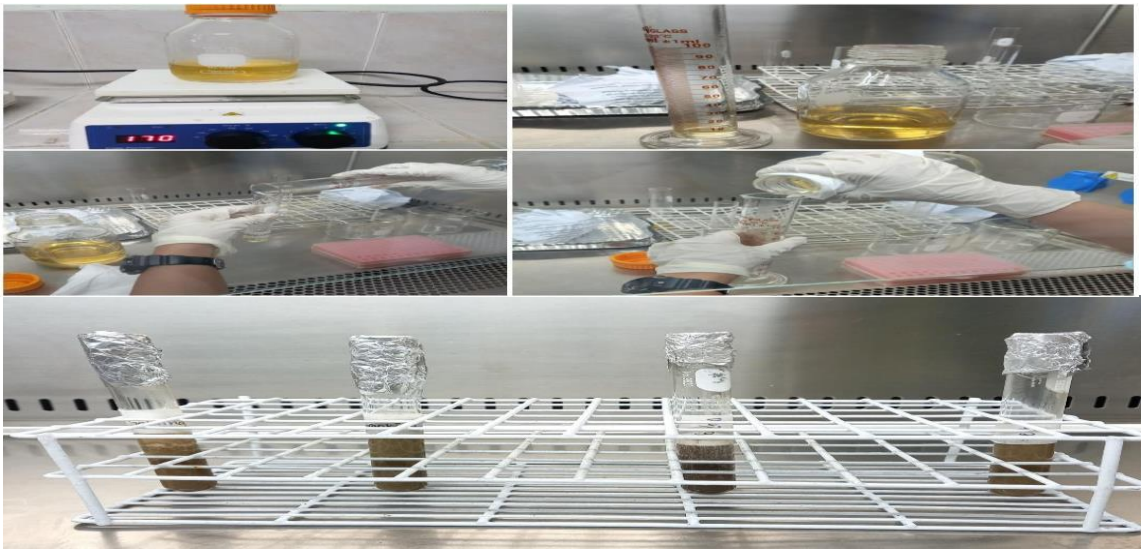

Figure 7. Prepared and Measured 10mL Nutrient Broth with Indian Oil Sardine Gut from Dried, Salted, and Smoked

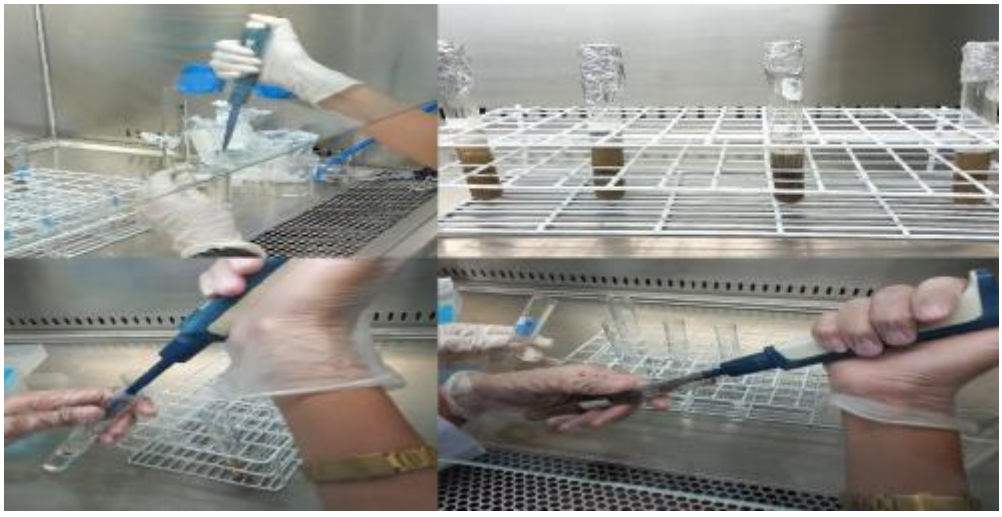

Figure 8. Performed 10-fold serial dilution from 10^1 to10^6

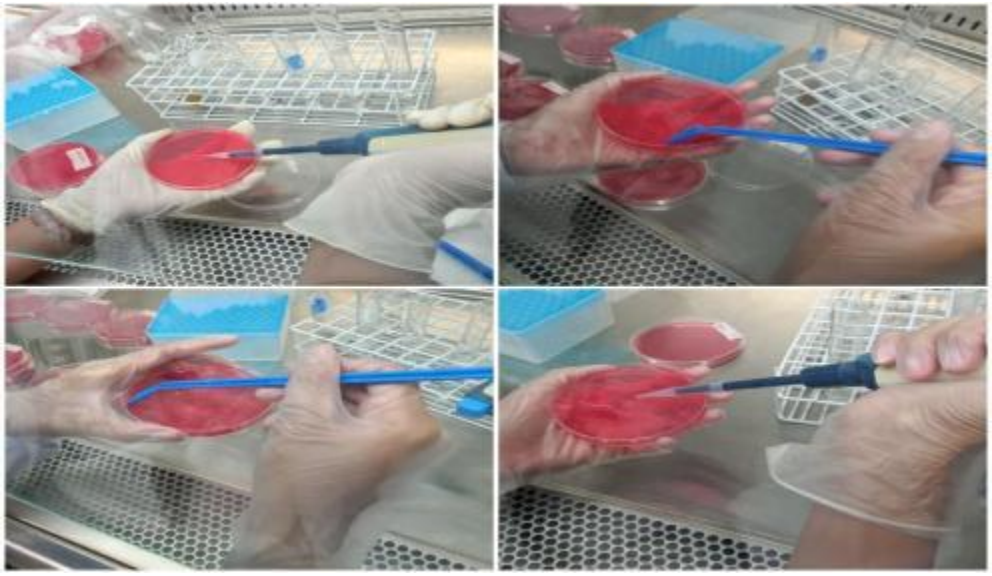

Figure 9. Spread Plate Techniqu

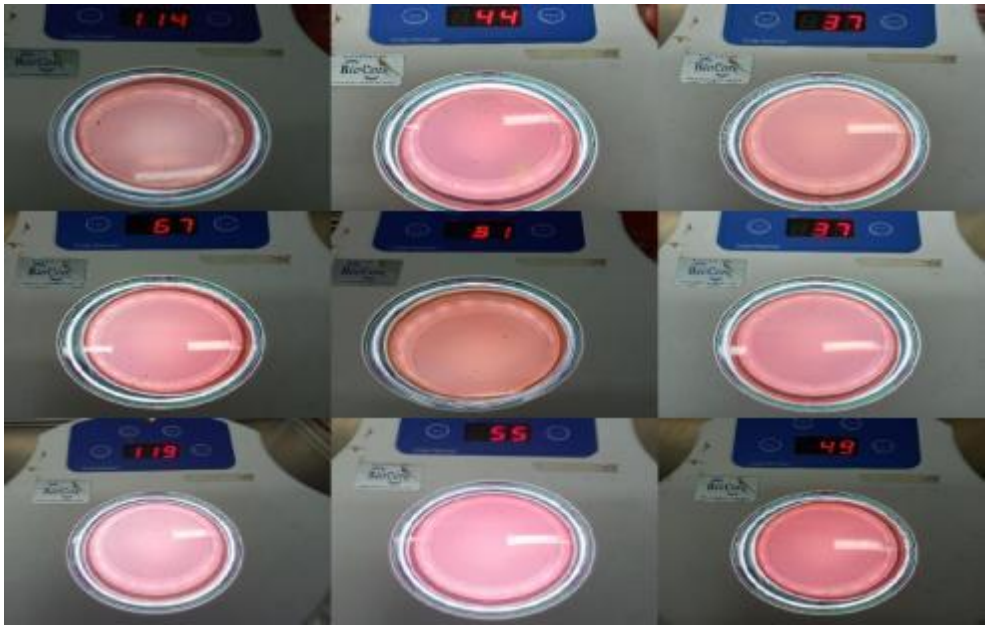

Figure 10. Counting Colony-Forming Units (CFUs)- in Drying

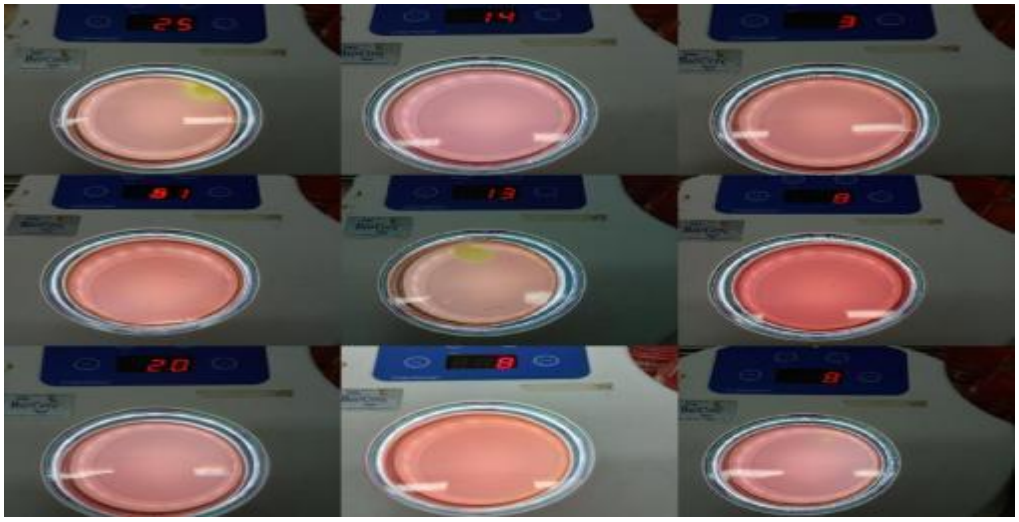

Figure 11. Counting Colony-Forming Units (CFUs)-in Salting

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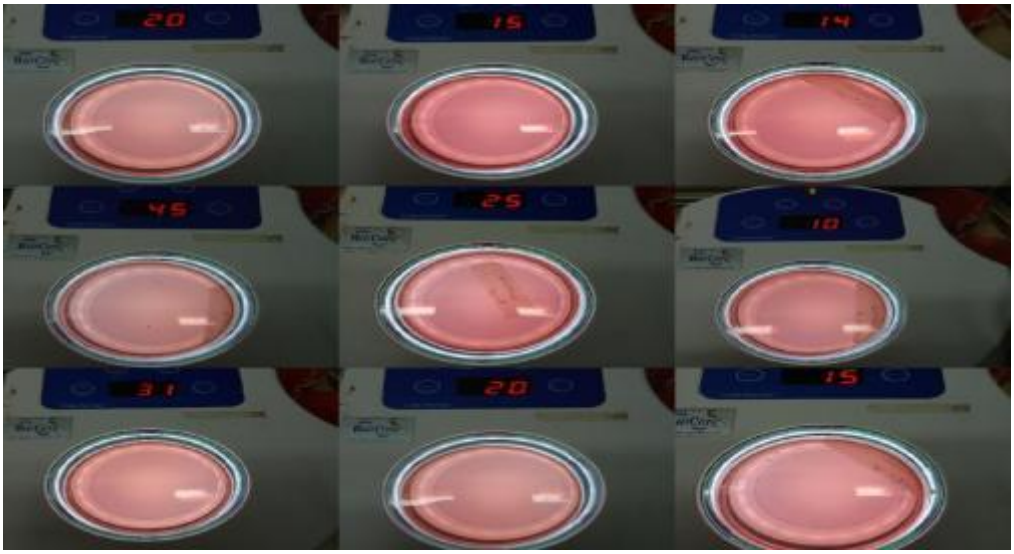

Figure 12. Counting Colony-Forming Units (CFUs)-in Smoking

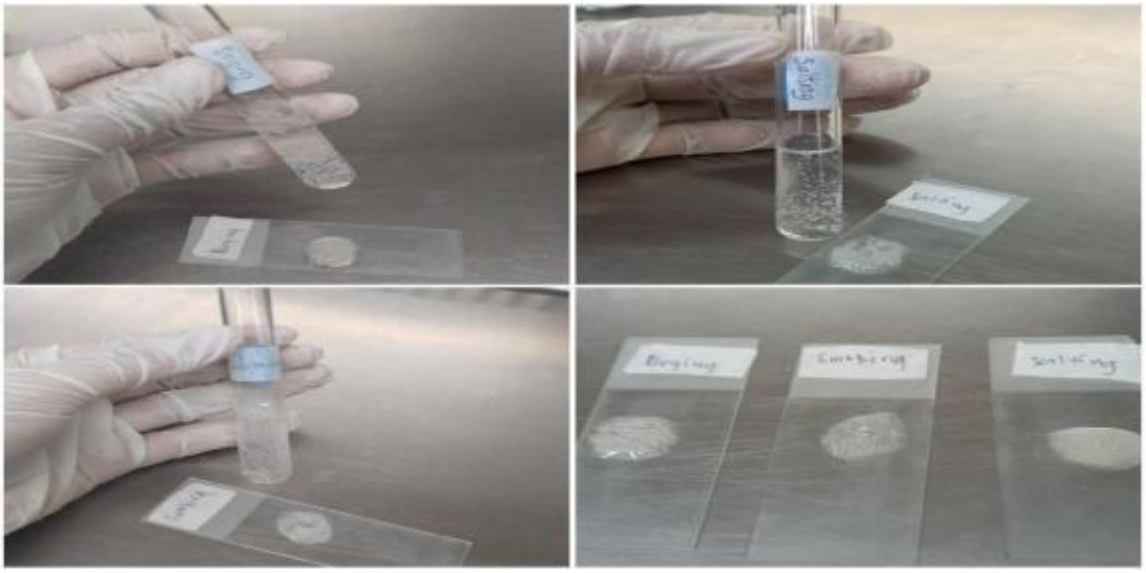

Figure 13. Catalase Test of Three (3) Preservation Techniques- Drying, Salting, and Smoking