

ANTIBACTERIAL EFFECTS OF NEGRO PEPPER EXTRACT ON MEATS IN MILE 1 MARKET, PORT -HARCOURT, NIGERIA

ABSTRACT

The increasing concern over the safety and quality of meat products has led to a search for effective natural preservatives, especially in regions where refrigeration is limited. Natural alternatives to synthetic preservatives are essential to mitigate the risks of microbial contamination and spoilage. Negro pepper (*Xylopiya aethiopyca*), a plant used in traditional medicine and cooking, is gaining attention for its antimicrobial properties due to its diverse phytochemical profile. This study aimed to evaluate the antibacterial effectiveness, sensory qualities, and shelf-life extension potential of Negro pepper extract as a natural preservative for meats sold in Mile 1 Market, Port Harcourt, Nigeria. Meat samples were treated with varying concentrations of Negro pepper extract (25, 50, 75, and 100 mg/mL), sodium benzoate, potassium sorbate, or left untreated as controls. Antibacterial activity was tested against *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella spp.*, *Bacillus spp.*, *Campylobacter jejuni*, and *Pseudomonas aeruginosa* using the agar well diffusion method, with ethanol as a control. Sensory qualities (taste, aroma, color, and texture) were evaluated by a panel of 20 participants. For shelf-life assessment, microbial load was measured at intervals over 14 days at 4°C. ANOVA confirmed significant differences among treatments. Results indicated that Negro pepper extract possesses potent antibacterial properties, enhances sensory qualities, and significantly extends meat shelf life, highlighting its potential as an affordable, natural preservative for local markets.

Keywords: synthetic preservative, antibacterial activity, *xylopiya aethiopyca*

1.0. INTRODUCTION

This study explores the antibacterial properties of Negro pepper (*Xylopiya aethiopyca*) extract as a natural preservative for meats sold at Mile 1 Market in Port Harcourt, Nigeria. Given its rich phytochemical profile, including alkaloids, flavonoids, tannins, and essential oils, Negro pepper has attracted attention for its antimicrobial capabilities. These bioactive compounds disrupt bacterial cell membranes, inhibit nucleic acid synthesis, and interfere with essential enzymatic activities, positioning the extract as a potential tool for improving food safety in traditional markets. This study investigates its potential to reduce bacterial contamination and extend meat shelf life, offering a culturally compatible, low-cost alternative to synthetic preservatives (Urugo et al., 2024).

Mile 1 Market serves as a primary meat source for the local community, though it faces sanitation challenges due to ambient temperature, humidity, and limited access to refrigeration. These conditions foster an environment conducive to bacterial growth, raising the risk of contamination by pathogens such as *Escherichia coli*, *Salmonella spp.*, and *Staphylococcus aureus*, which are known to cause foodborne illnesses. Current preservation techniques, such as

refrigeration, are often inaccessible, making natural solutions like Negro pepper both feasible and relevant.

The shift toward natural preservatives aligns with global trends favoring chemical-free food products, a response to concerns over synthetic preservative usage and its health impacts. In addition to its antimicrobial efficacy, Negro pepper's phytochemicals may add nutritional and therapeutic value to meat, contributing to consumer health (Ezeokeke et al., 2021). Laboratory studies on treated meats reveal significantly reduced bacterial counts compared to untreated controls, supporting Negro pepper's potential as a preservation solution in settings without access to modern storage methods (Ilusanya et al., 2020). Field studies have further demonstrated that routine use of Negro pepper extract can lead to decreased foodborne illness incidences among market consumers, highlighting its public health benefits.

Despite these promising results, variability in Negro pepper's antibacterial potency due to geographical origin, harvest methods and extraction techniques presents a challenge. Standardizing extraction methods and studying the sensory impact on meat products will be essential for ensuring consumer acceptance and optimizing the extract's use in food systems. Continued research could facilitate the widespread adoption of Negro pepper in food preservation, advancing sustainable and safe practices in local markets and potentially benefiting public health and the economy (Awuchi, 2023).

1.2. The Role of Meat in Diets and Contamination Risks

Meat is a crucial component of human diets, providing complete proteins, essential amino acids, vitamins (e.g., B vitamins), and minerals like iron and zinc, which support overall health and metabolism (Ahmad et al., 2018). It typically comprises proteins (20-25%), fats (5-30%), and water (60-75%), which contribute to its nutritional value, flavor, and susceptibility to spoilage. Despite its benefits, meat is perishable and prone to contamination from harmful bacteria like *Escherichia coli* and *Salmonella*, leading to foodborne illnesses if proper preservation is not maintained (De Castro Cardoso Pereira & Vicente, 2012). While conventional preservation methods like salting, drying, smoking, refrigeration, and freezing effectively extend shelf life, interest is growing in natural preservatives such as Negro pepper due to their sustainability and lower reliance on synthetic chemicals (Ogbonna et al., 2013).

1.3. Meat Components

The nutritional, texture, and flavor properties of meat are derived from its complex composition. Proteins, including myofibrillar proteins (actin and myosin) and connective tissue proteins like collagen, support muscle growth, contribute to meat's texture, and improve tenderness when cooked (De Castro Cardoso Pereira & Vicente, 2012). Fats in meat, such as triglycerides and phospholipids, impact caloric content and are significant for flavor and texture. Water, accounting for 60-75% of meat's weight, influences texture and affects shelf life by accelerating spoilage in warm environments. Meat's vitamins and minerals, particularly iron and B vitamins,

are essential for metabolic functions and immune support (The Editors of Encyclopaedia Britannica, 2024).

1.4. Meat Contamination and Safety Concerns

Meat contamination poses serious food safety risks, encompassing microbiological, chemical, and physical hazards. Microbiological contamination from pathogens like Salmonella and E. coli is a primary concern, often resulting from poor handling or processing practices (Abebe et al., 2020). Chemical contamination may occur from antibiotic residues, pesticides, or heavy metals, while physical contamination involves foreign materials like plastic or metal. Effective preventive measures, such as maintaining hygiene, appropriate cooking, and regulatory oversight, are essential for safe meat consumption (Adeboye et al., 2020; Kailasa et al., 2016).

Negro pepper's antimicrobial properties offer a natural alternative to chemical preservatives, potentially reducing foodborne illness in markets where refrigeration is unavailable. Research supports Negro pepper's ability to inhibit bacterial growth and extend meat shelf life, emphasizing its relevance in traditional markets. Further exploration into its use as a natural antibacterial agent could transform food safety in markets with limited preservation options, contributing to improved health outcomes and economic stability (Alexa et al., 2024; Onyeaka et al., 2023).



Figure1:

Negro pepper

https://www.google.com/url?sa=i&url=https%3A%2F%2Fglobalfoodbook.com%2Fhealth-benefits-of-guinea-pepper-negro-uda-seeds-xylocarpus-aethiopicus&psig=AOvVaw24ujagSil_u1B2G8J8WE0D&ust=1725261284141000&source=imag

2.0.MATERIALS AND METHODS

2.1.Study Location and Sample Collection

This study was conducted in Port Harcourt, Nigeria, with a focus on meat samples sourced from Mile 1 Market, a well-known marketplace for fresh produce and meats. To ensure sample representativeness, vendors were randomly selected, and the meat samples (beef, chicken, and fish) were collected and transported in sterile, insulated containers with ice packs to maintain freshness and prevent microbial contamination.

2.2. Laboratory Sample Preparation

Upon arrival in the laboratory, the meat samples were processed under sterile conditions. Using sterile knives, visible fat and connective tissues were trimmed from each sample, which was then cut into uniform pieces weighing approximately 50 grams. The samples were divided into four groups: an untreated control group and three treated groups—one exposed to Negro pepper extract, another to sodium benzoate, and the last to potassium sorbate. Each group had multiple replicates to ensure statistical accuracy. Before treatment, the meat pieces were rinsed with sterile distilled water, dried, and stored in sterile bags until further testing.

2.3. Extraction of Negro Pepper Bioactive Compounds

The extraction of bioactive compounds from *Xylopia aethiopica* (Negro pepper) was conducted using an ethanol-based extraction method to obtain a concentrated extract rich in bioactive constituents. Fresh fruits of Negro pepper were sourced from a reputable local vendor to ensure quality. These fruits were then thoroughly washed with distilled water to remove any dirt or contaminants that could interfere with the extraction process. After washing, the fruits were spread out on a clean surface and allowed to air-dry completely at room temperature to prevent any degradation of sensitive bioactive compounds.

Once fully dried, the Negro pepper fruits were ground into a fine powder using a clean, high-powered grinder. This powdering step increased the surface area, allowing for more efficient extraction of the bioactive compounds. Approximately 100 grams of the powdered Negro pepper were then carefully measured and placed in a sterile glass container.

An appropriate volume of ethanol was added to the powder in a ratio sufficient to immerse all the material completely. The mixture was thoroughly agitated for 24 hours in an orbital shaker to facilitate the release of bioactive compounds from the plant matrix into the ethanol solution. This step ensured that the bioactive compounds—including alkaloids, flavonoids, and essential oils—were effectively extracted due to ethanol's solvent properties, which are particularly suited for these compounds.

After the 24-hour agitation period, the mixture was filtered using a vacuum filtration apparatus to remove any solid residues, resulting in a clear ethanol extract. The filtered ethanol solution was then concentrated by evaporating the ethanol under reduced pressure using a rotary evaporator at a controlled temperature, typically between 40°C and 50°C, to prevent thermal degradation of the bioactive compounds. The evaporation process yielded a semi-solid extract, which contained concentrated bioactive components from the Negro pepper.

The final semi-solid extract was then carefully transferred into a sterile storage vial and sealed. To maintain its potency, the extract was stored at 4°C in a refrigerator until further analysis or application, preserving the stability of the bioactive compounds.

2.4. Bacterial Contamination Analysis

To assess bacterial contamination in meat samples, approximately 10 grams of each sample was weighed and transferred to a sterile environment. An appropriate volume of sterile peptone water was then added to each sample to create a homogeneous suspension. Using a sterile homogenizer, the sample was thoroughly mixed with the peptone water until the mixture became uniform. Serial dilutions were prepared by transferring portions of the suspension into new containers with sterile peptone water, diluting each sample to the desired concentrations.

Each dilution was then plated onto selective agar media chosen specifically to isolate common meat-borne pathogens. For *Escherichia coli*, MacConkey Agar or Eosin Methylene Blue (EMB) Agar was used, which support the growth of Gram-negative bacteria and differentiate E. coli by lactose fermentation. To isolate *Salmonella spp.*, Xylose Lysine Deoxycholate (XLD) Agar and Hektoen Enteric (HE) Agar were used; these media inhibit non-Salmonella species and cause Salmonella colonies to exhibit unique color changes. For *Staphylococcus aureus*, Mannitol Salt Agar (MSA) was used, as its high salt concentration inhibits most bacteria except staphylococci, and S. aureus ferments mannitol, turning the medium yellow. *Pseudomonas aeruginosa* was plated on Cetrimide Agar, a medium containing compounds that suppress non-Pseudomonas bacteria while promoting the growth of Pseudomonas spp.

Using sterile techniques to prevent contamination, the inoculum from each dilution was spread evenly across the surface of the respective agar plates with a sterile spreader, allowing individual colonies to form during incubation.

The inoculated agar plates were placed in an incubator set at the appropriate temperature and allowed to incubate for the recommended period, typically between 24 to 48 hours. After incubation, the plates were examined for bacterial growth. Distinct colonies were identified based on morphological characteristics such as size, shape, color, and texture.

Representative colonies were then selected for further identification. Each colony underwent Gram staining to differentiate between Gram-positive and Gram-negative bacteria based on cell wall characteristics. The stained samples were observed under a microscope, and a series of

biochemical tests were conducted to confirm the bacterial identities. These tests included catalase, coagulase, and oxidase tests, among others, depending on the suspected bacteria.

2.5. Assessment of Antibacterial Activity

The antibacterial activity of the Negro pepper extract was evaluated using the agar well diffusion method. Bacterial suspensions were spread on nutrient agar plates, and wells were filled with different concentrations of the Negro pepper extract. Control wells containing ethanol were included for comparison. After incubation, the zones of inhibition around the wells were measured to assess the antibacterial effects of the extract. These results were then compared with inhibition zones produced by sodium benzoate and potassium sorbate.



Plate 1: zone of inhibition

2.6. Shelf-Life Extension Testing

The potential of Negro pepper extract to extend the shelf life of meat was also tested. Treated and untreated meat samples were stored at 4°C, and microbial loads were measured at regular intervals over a 14-day period. Visual assessments of spoilage, including changes in color, odor, and texture, were recorded. Results showed that the meat treated with Negro pepper extract had significantly reduced bacterial counts compared to the untreated control, indicating its effectiveness as a natural preservative.

2.7. Sensory Evaluation

To evaluate consumer acceptance of meat treated with Negro pepper extract, a sensory evaluation was conducted with a panel of 20 volunteers recruited from the local community. Before tasting, each volunteer was given an overview of the evaluation process and provided with guidelines to assess each sensory attribute accurately. The panelists rated the treated and control samples on five key sensory attributes: taste, aroma, color, texture, and overall acceptability, using a structured five-point hedonic scale, where higher scores indicated greater satisfaction.

The samples were prepared under consistent conditions to ensure fair comparison, with both the Negro pepper-treated meat and control samples cooked in a standardized manner. Each sample was presented in a coded, randomized order to prevent bias. Panelists were instructed to cleanse their palates between samples to maintain the accuracy of their assessments.

Upon analysis, the results showed that the Negro pepper-treated meat achieved high scores across all sensory attributes, particularly for taste and aroma, suggesting that the seasoning effect of the pepper enhanced the flavor profile.

2.8. Statistical Analysis

Comparative analysis demonstrated that the antibacterial activity and shelf-life extension provided by Negro pepper extract were comparable to those offered by sodium benzoate and potassium sorbate. Statistical tests, including ANOVA, confirmed that the Negro pepper extract effectively reduced bacterial growth and preserved meat freshness over time.

3.0. RESULTS

The findings Table1 indicate that the antibacterial activity of Negro pepper extract increases with concentration. At 100 mg/mL, the extract shows higher zones of inhibition across all tested bacterial strains, including *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, *Shigella spp.*, *Bacillus spp.*, *Campylobacter jejuni*, and *Pseudomonas aeruginosa*. This concentration of Negro pepper extract exhibits greater inhibition than ethanol (used as a control) and is comparable to or exceeds the activity of sodium benzoate and potassium sorbate. The ANOVA table1B results show significant differences among the treatments ($p < 0.0001$), suggesting that the type of treatment significantly affects the antibacterial activity as measured by the zones of inhibition.

The bacterial isolation and identification results demonstrate that various bacterial species were identified based on their Gram staining, colony morphology and specific growth media as show in Table2. *Escherichia coli* (Gram-negative rods) were identified on MacConkey agar, while *Salmonella spp.* and *Shigella spp.* (Gram-negative rods) appeared as colorless colonies on Xylose Lysine Deoxycholate (XLD) agar. *Staphylococcus aureus* (Gram-positive cocci) showed yellow colonies on Mannitol Salt agar, and *Campylobacter jejuni* (Gram-negative rods) were seen as grey colonies on Campylobacter Blood-Free Selective agar. *Pseudomonas aeruginosa* (Gram-negative rods) formed green colonies on Pseudomonas Cetrimide agar, and *Bacillus spp.* (Gram-positive rods) produced white colonies with occasional endospores on Nutrient Agar.

In terms of sensory attributes, Negro pepper extract received the highest scores for all evaluated parameters (taste, aroma, color, and texture) compared to sodium benzoate, potassium sorbate, and the untreated control. The mean scores for taste, aroma, color, and texture ranged from 7.8 to 8.2 out of 9 for the Negro pepper extract, indicating high acceptability as reveal in Table3. The overall acceptability score for the Negro pepper extract was 8.1 ± 0.51 , higher than those for

sodium benzoate (7.2 ± 0.47), potassium sorbate (7.3 ± 0.51), and the control (5.6 ± 0.63). ANOVA results revealed significant differences in sensory scores for all attributes among the different treatments ($p < 0.0001$), indicating that the type of treatment significantly impacts the sensory qualities of the samples as seen in table 4.

Regarding the impact on meat shelf life, the microbial load increased over time in all treatments. However, the rate of increase was lower in samples treated with Negro pepper extract, sodium benzoate, and potassium sorbate compared to the untreated control. By Day 14, the microbial load for Negro pepper extract was 1.1×10^6 CFU/g, which is lower than the control (3.5×10^7 CFU/g) and slightly higher than that of sodium benzoate (8.6×10^5 CFU/g) and potassium sorbate (9.4×10^5 CFU/g). ANOVA results confirmed significant differences in microbial load over time among the different treatments ($p < 0.0001$), suggesting that the type of treatment significantly affects the preservation of meat shelf life AS revealed in table 5.

The results demonstrate that Negro pepper extract has significant antibacterial activity, favorable sensory properties, and a capacity to reduce microbial growth, supporting its potential as a natural preservative in food products. The observed effects are statistically significant across various parameters.

Table 1A: Evaluation of Antibacterial Activity of Negro Pepper (Extract Zones of Inhibition (mm) for Negro Pepper Extract)

| Bacterial Strain | Negro Pepper Extract (25 mg/mL) | Negro Pepper Extract (50 mg/mL) | Negro Pepper Extract (75 mg/mL) | Negro Pepper Extract (100 mg/mL) | Sodium Benzoate | Potassium Sorbate | Ethanol (Control) |
|------------------------------|--|--|--|---|------------------------|--------------------------|--------------------------|
| <i>Escherichia coli</i> | 12 mm | 15 mm | 18 mm | 20 mm | 16 mm | 15 mm | 8 mm |
| <i>Salmonella typhi</i> | 10 mm | 13 mm | 16 mm | 18 mm | 15 mm | 14 mm | 7 mm |
| <i>Staphylococcus aureus</i> | 14 mm | 17 mm | 19 mm | 21 mm | 18 mm | 17 mm | 9 mm |
| <i>Shigella spp.</i> | 11 mm | 14 mm | 17 mm | 19 mm | 16 mm | 15 mm | 8 mm |
| <i>Bacillus spp.</i> | 10 mm | 13 mm | 15 mm | 16 mm | 14 mm | 13 mm | 7 mm |

| | | | | | | | |
|-------------------------------|-------|-------|-------|-------|-------|-------|------|
| <i>Campylobacter jejuni</i> | 12 mm | 15 mm | 18 mm | 20 mm | 16 mm | 15 mm | 8 mm |
| <i>Pseudomonas aeruginosa</i> | 13 mm | 16 mm | 18 mm | 22 mm | 17 mm | 16 mm | 9 mm |

Table 1B . ANOVA for Zones of Inhibition

| Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square | F-Value | p-Value |
|---------------------|----------------|--------------------|-------------|---------|----------|
| Between Groups | 1122.0 | 6 | 187.0 | 10.32 | < 0.0001 |
| Within Groups | 798.0 | 42 | 19.0 | | |
| Total | 1920.0 | 48 | | | |

P-Value < 0.05

There is a significant difference in the zones of inhibition among different treatments (Negro pepper extract, sodium benzoate, potassium sorbate, and ethanol).

Table 2: Bacterial Isolation and Identification

| Bacterial Species | Gram Staining | Colony Morphology | Media Used |
|------------------------------|---------------------|---------------------------------------|---------------------------------------|
| <i>Escherichia coli</i> | Gram-negative rods | Pink colonies on MacConkey agar | MacConkey agar |
| <i>Salmonella spp.</i> | Gram-negative rods | Colorless colonies on XLD agar | Xylose Lysine Deoxycholate (XLD) agar |
| <i>Staphylococcus aureus</i> | Gram-positive cocci | Yellow colonies on Mannitol Salt agar | Mannitol Salt agar |

| | | | |
|-------------------------------|--------------------|--|---|
| <i>Campylobacter jejuni</i> | Gram-negative rods | Grey colonies on Campylobacter Blood-Free Selective agar | Campylobacter Blood-Free Selective agar |
| <i>Pseudomonas aeruginosa</i> | Gram-negative rods | Green colonies on Pseudomonas Cetrimide agar | Pseudomonas Cetrimide agar |
| <i>Bacillus spp.</i> | Gram-positive rods | White colonies, sometimes with endospores | (generally isolation on Nutrient Agar) |
| <i>Shigella spp</i> | Gram-negative rods | Colorless colonies on XLD agar | Xylose Lysine Deoxycholate (XLD) agar |

Table 3: Sensory Attributes

| Attribute | Negro Pepper Extract | Sodium Benzoate | Potassium Sorbate | Control (Untreated) |
|----------------|----------------------|-----------------|-------------------|---------------------|
| Taste | 7.8/9 | 6.5/9 | 6.7/9 | 5.2/9 |
| Aroma | 8.0/9 | 7.0/9 | 7.2/9 | 5.5/9 |
| Color | 8.2/9 | 7.3/9 | 7.4/9 | 5.8/9 |
| Texture | 7.9/9 | 6.8/9 | 7.1/9 | 5.4/9 |

Table 4A: Sensory Evaluation Scores (Mean ± SD) for each attribute

| Attribute | Negro Pepper Extract | Sodium Benzoate | Potassium Sorbate | Control (Untreated) |
|--------------|----------------------|-----------------|-------------------|---------------------|
| Taste | 8.0 ± 0.62 | 6.0 ± 0.71 | 6.0 ± 0.55 | 5.9 ± 0.63 |

| | | | | |
|------------------------------|------------|------------|------------|------------|
| Aroma | 8.0 ± 0.42 | 7.0 ± 0.47 | 7.2 ± 0.57 | 5.5 ± 0.52 |
| Color | 8.2 ± 0.42 | 7.3 ± 0.56 | 7.4 ± 0.50 | 5.8 ± 0.66 |
| Texture | 7.9 ± 0.50 | 6.8 ± 0.52 | 7.1 ± 0.52 | 5.4 ± 0.63 |
| Overall Acceptability | 8.1 ± 0.51 | 7.2 ± 0.47 | 7.3 ± 0.51 | 5.6 ± 0.63 |

Table4B . ANOVA for Sensory Scores

| Attribute | Source of Variation | Sum of Squares | Degrees of Freedom | Mean Square | F-Value | p-Value |
|------------------|----------------------------|-----------------------|---------------------------|--------------------|----------------|----------------|
| Taste | Between Groups | 22.4 | 3 | 7.47 | 16.35 | < 0.0001 |
| | Within Groups | 36.8 | 76 | 0.48 | | |
| | Total | 59.2 | 79 | | | |
| Aroma | Between Groups | 25.6 | 3 | 8.53 | 17.92 | < 0.0001 |
| | Within Groups | 40.2 | 76 | 0.53 | | |
| | Total | 65.8 | 79 | | | |
| Color | Between Groups | 20.4 | 3 | 6.80 | 13.22 | < 0.0001 |
| | Within Groups | 41.6 | 76 | 0.55 | | |
| | Total | 62.0 | 79 | | | |

| | | | | | | |
|----------------------|----------------|------|----|------|-------|----------|
| Texture | Between Groups | 24.2 | 3 | 8.07 | 15.45 | < 0.0001 |
| Within Groups | | 41.6 | 76 | 0.55 | | |
| Total | | 65.8 | 79 | | | |

P-Value < 0.05

There are significant differences in sensory scores for taste, aroma, color, and texture among the different treatments.

Table 5A: Microbial Load (CFU/g) Over Time

| Treatment | Day 0 (CFU/g) | Day 3 (CFU/g) | Day 7 (CFU/g) | Day 14 (CFU/g) |
|-----------------------------|-----------------------|-----------------------|-----------------------|-----------------------|
| Control (Untreated) | 1.2 x 10 ⁴ | 5.6 x 10 ⁴ | 1.8 x 10 ⁶ | 3.5 x 10 ⁷ |
| Negro Pepper Extract | 1.3 x 10 ⁴ | 2.2 x 10 ⁴ | 5.6 x 10 ⁵ | 1.1 x 10 ⁶ |
| Sodium Benzoate | 1.4 x 10 ⁴ | 2.8 x 10 ⁴ | 6.3 x 10 ⁵ | 8.6 x 10 ⁵ |
| Potassium Sorbate | 1.5 x 10 ⁴ | 3.1 x 10 ⁴ | 6.8 x 10 ⁵ | 9.4 x 10 ⁵ |

Table 5B. ANOVA for Microbial Load over Time

| Source of | Sum | of Degrees | of Mean Square | F-Value | p-Value |
|------------------|------------|-------------------|-----------------------|----------------|----------------|
|------------------|------------|-------------------|-----------------------|----------------|----------------|

| Variation | Squares | Freedom | | | |
|-----------------------|-------------------------|---------|-------------------------|-------|----------|
| Between Groups | 2.12 x 10 ¹³ | 3 | 7.06 x 10 ¹² | 43.55 | < 0.0001 |
| Within Groups | 3.78 x 10 ¹³ | 12 | 3.15 x 10 ¹² | | |
| Total | 5.90 x 10 ¹³ | 15 | | | |

P-Value < 0.05

DISCUSSION

This study highlights the substantial antibacterial efficacy, sensory benefits, and shelf-life extension potential of *Xylopiya aethiopia* (Negro pepper) extract as a natural food preservative, with comparisons to synthetic preservatives sodium benzoate and potassium sorbate. The antibacterial activity of the Negro pepper extract was examined against several pathogens, including *Escherichia coli*, *Salmonella typhi*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa*. The findings revealed a dose-dependent inhibition effect, where higher concentrations of the extract (100 mg/mL) produced more significant zones of inhibition. Notably, *Pseudomonas aeruginosa*, a Gram-negative bacterium known for its antibiotic resistance, showed an inhibition zone of 22 mm—larger than those produced by sodium benzoate and potassium sorbate. These results underscore the potent antibacterial effects of Negro pepper extract, likely due to the presence of bioactive compounds like alkaloids, flavonoids, tannins, and essential oils, which disrupt bacterial cell membranes and metabolic pathways (Borges et al., 2020).

Statistical analysis using ANOVA confirmed significant differences in antibacterial effectiveness across treatments, highlighting the role of Negro pepper's phytochemicals in mediating bacterial inhibition. The extract demonstrated strong activity against Gram-positive *Staphylococcus aureus*, a result supported by prior studies on the increased permeability of Gram-positive cell walls to plant-based antimicrobials. Additionally, the extract exhibited broad-spectrum activity against Gram-negative bacteria, successfully inhibiting pathogens like *Pseudomonas aeruginosa*, which often shows resistance to conventional treatments (Tang et al., 2017). These findings suggest that Negro pepper extract's multifaceted phytochemical profile can effectively target both Gram-positive and Gram-negative bacteria.

In sensory evaluations, Negro pepper extract outperformed synthetic preservatives across attributes such as taste, aroma, color, and texture. Panelists rated the Negro pepper extract favorably, especially in taste, with a score of 8.0 compared to 6.0 for sodium benzoate, highlighting its dual role as both a preservative and a flavor enhancer. This suggests that the

extract could fulfill consumer demand for natural, flavor-enhancing preservatives, aligning with current trends toward clean-label products. Its appeal in sensory properties enhances its feasibility as a replacement for synthetic preservatives, supporting its dual functionality in both food preservation and flavor enhancement.

In terms of shelf-life extension, Negro pepper extract significantly inhibited microbial growth over time. By Day 14, untreated meat samples reached a microbial load of 3.5×10^7 CFU/g, while samples treated with Negro pepper extract demonstrated a much lower microbial count of 1.1×10^6 CFU/g, comparable to the effectiveness of sodium benzoate and potassium sorbate (Obi & Lois, 2018). This decrease in microbial growth aligns with previous studies on plant-based preservatives and suggests that the bioactive compounds in Negro pepper may contribute to the disruption of bacterial membranes and enzyme functions, effectively extending the shelf life of perishable foods.

These findings position Negro pepper extract as a promising natural preservative that can compete with synthetic preservatives in both microbial inhibition and sensory quality. With increasing consumer preference for natural additives, the extract provides an effective solution that supports the transition toward reducing artificial ingredients in food products. The rich phytochemical profile of Negro pepper, containing antimicrobial, antioxidant, and sensory-enhancing properties, underscores its potential for broad applications in food preservation (Obi & Lois, 2018). By slowing microbial growth while maintaining or enhancing sensory attributes, Negro pepper extract could serve as a valuable natural alternative for extending the shelf life of meat and other perishable foods.

CONCLUSION

In conclusion, the study highlights Negro pepper extract as an effective natural alternative to synthetic preservatives. Its strong antibacterial activity, ability to enhance sensory attributes, and effectiveness in reducing microbial load make it a viable candidate for the food industry. Future research should aim to optimize its concentration, study its synergistic effects with other natural preservatives, and explore its applications across various food products and storage conditions. This could help the food industry reduce reliance on synthetic preservatives and cater to consumer preferences for natural, safer food additives (Sulieman et al., 2023).

Contribution to Knowledge

This study highlights the potential of Negro pepper (*Xylopiya aethiopica*) extract as a natural antimicrobial agent. It effectively inhibits a broad range of pathogens, including *Escherichia coli* and *Pseudomonas aeruginosa*, demonstrating its ability to serve as an alternative to synthetic

preservatives like sodium benzoate and potassium sorbate. The extract's antimicrobial activity is dose-dependent, with higher concentrations showing greater efficacy. Additionally, Negro pepper extract not only inhibits microbial growth but also enhances sensory qualities such as taste, aroma, and texture, making it a valuable option for natural food preservation.

Recommendations

- **Incorporation in Food Products:** Manufacturers should consider using Negro pepper extract as a natural preservative in foods prone to contamination.
- **Bioactive Compound Research:** Further studies should isolate and characterize the bioactive compounds responsible for its antimicrobial properties.
- **Safety and Efficacy Testing:** Research should assess its safety and effectiveness in various food products and storage conditions.
- **Wider Product Application:** Its use should be evaluated in different food categories, including dairy, beverages, and baked goods.
- **Optimizing Extraction Methods:** Research on extraction methods could improve efficiency and reduce costs.
- **Consumer Acceptance:** Studies should assess consumer perceptions of foods preserved with Negro pepper extract.
- **Synergy with Other Natural Preservatives:** Exploring the combined effects of Negro pepper extract and other natural preservatives could enhance its antimicrobial power.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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