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Effects of Natural and Synthetic Preservatives on the Microbial, Physicochemical, and Shelf-Life Characteristics of Dried Beef Under Ambient Storage Conditions

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ABSTRACT (10 PT)

This study investigated the effects of natural (ginger and garlic) and synthetic (potassium sorbate and sodium nitrite) preservatives on the microbial quality, physicochemical quality, and shelf life of dried beef stored under ambient conditions. Over a 9-day storage period, samples were analyzed for total bacterial counts, coliforms, staphylococcal and fungal counts, moisture content, and total volatile base nitrogen (TVB-N). The results revealed that untreated samples deteriorated rapidly, showing high microbial proliferation (total viable count increasing from 0.00 to 5.5 log CFU/g) and significant biochemical spoilage, with TVB-N rising from 7.25 to 24.60 mg N/100 g. In contrast, treated samples, particularly those preserved with potassium sorbate and sodium nitrite, showed markedly lower microbial counts (3.55 to 5.5 log CFU/g), controlled TVB-N values (6.70 to 15.00 mg N/100 g), and reduced moisture loss (30.40 ± 0.12% to 27.00 ± 0.18%) compared to the control. Microorganisms isolated revealed Bacillus subtilis, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa as the dominant bacteria, while Aspergillus niger, Saccharomyces cerevisiae, and Candida spp. were the major fungi. Natural preservatives (ginger and garlic) exhibited antimicrobial activity, especially when combined, while synthetic preservatives demonstrated superior inhibitory and stabilizing effects ($p < 0.05$). Overall, the study concludes that both natural and synthetic preservatives significantly enhanced the safety and shelf life of dried beef, with synthetic treatments proving most effective. Nonetheless, natural preservatives such as ginger and garlic represent promising, health-friendly alternatives that can be optimized for sustainable meat preservation.

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1. INTRODUCTION

The desire to provide meat products that combine superior quality, affordability, eco-friendliness, and consumer appeal has driven meat processors, distributors, retailers, and industrialists to continuously improve meat preservation strategies [1]. Meat remains a vital source of animal-based nutrition for humans, supplying high-quality protein and essential nutrients. In recent years, global consumer demand for meat has increased considerably due to its desirable sensory attributes and nutritional benefits [2]. However, this growing demand has also intensified concerns regarding meat safety and shelf-life stability.

Microbial contamination of meat is a major global food safety issue that affects both consumers and the food industry by reducing shelf life and increasing the risk of foodborne diseases [3]. Meat is highly perishable because of its energy-dense nutrients, high water activity, and near-neutral pH, all of which favor microbial growth [4]. Contamination can occur at multiple stages, beginning at the abattoir and continuing through deboning, processing, handling, storage, and distribution. The initial mesophilic microflora of fresh meat typically ranges between 10^2 and 10^3 CFU cm^{-2} [5]. Further processing, especially into comminuted meat products, increases the risk of cross-contamination and microbial proliferation [3].

Ensuring microbial safety while extending the shelf life of meat products remains a critical challenge in the food industry. Conventional preservation methods largely depend on synthetic preservatives, which are effective but increasingly questioned due to potential health concerns and growing consumer preference for clean-label foods. At the same time, natural preservatives, particularly plant-derived spices, have gained attention for their antimicrobial potential and sensory benefits. However, their effectiveness varies depending on concentration, food matrix, and storage conditions [6].

Natural preservatives such as ginger (Zingiber officinale) and garlic (Allium sativum) have demonstrated significant antimicrobial and antioxidant properties. Ginger contains bioactive compounds, including gingerols, shogaols, and zingerone, which contribute to its preservative effects [7]. Studies have shown that ginger-based films and encapsulations improve preservation in meat, fish, and bakery products [8], while ginger and garlic combinations have reduced microbial counts in food systems during storage [9]. Despite these promising findings, variations in formulation and application limit their consistent adoption in meat preservation.

Similarly, garlic owes its antimicrobial efficacy to sulfur-containing compounds such as allicin, which forms when garlic is crushed [10]. Research has shown that garlic can reduce microbial growth and enhance oxidative stability in meat products [11], [12]. However, its strong flavor profile may affect consumer acceptability if not carefully formulated [13]. Meanwhile, pathogenic bacteria such as Salmonella typhi, Escherichia coli, and Staphylococcus aureus remain major etiological agents of meat-borne illnesses, posing persistent public health risks when preservation is inadequate [14].

Synthetic preservatives such as sodium nitrite (E250) and potassium sorbate (E202) remain widely used due to their proven antimicrobial effectiveness. Potassium sorbate inhibits microbial growth by disrupting cell membranes and enzyme systems and has shown effectiveness against yeasts, molds, and some bacteria [15], , [16]. Sodium nitrite plays a key role in inhibiting pathogens such as Clostridium botulinum, stabilizing color, and preventing lipid oxidation in cured meats [17]. However, health concerns and strict regulatory controls limit their application, particularly in traditional dried meat products such as kilishi, were storage conditions further influence safety and quality [18], [19].

Although both natural and synthetic preservatives have demonstrated effectiveness, there is limited comparative research evaluating their performance in dried beef products under ambient storage conditions. Factors such as temperature, humidity, light exposure, and packaging significantly influence spoilage rates and microbial growth [20]. Therefore, this study aims to evaluate and compare the effects of natural preservatives (garlic and ginger extracts) and synthetic preservatives (sodium nitrite and potassium sorbate) on the shelf life and microbial quality of dried beef stored at ambient temperature. The findings will contribute to the development of safer, consumer-friendly, and optimized meat preservation strategies.

2. METHODS

2.1. Research Stages

The research workflow involves systematically preparing and treating beef samples with natural (garlic, ginger) and synthetic (sodium nitrite, potassium sorbate) preservatives, followed by smoking, drying, cooling, packaging, and storage at ambient temperature. Samples are then analyzed at specific intervals for microbiological and physicochemical properties, and the resulting data are interpreted to assess preservative efficacy and optimize dried meat safety and quality (**Fig. 1**).

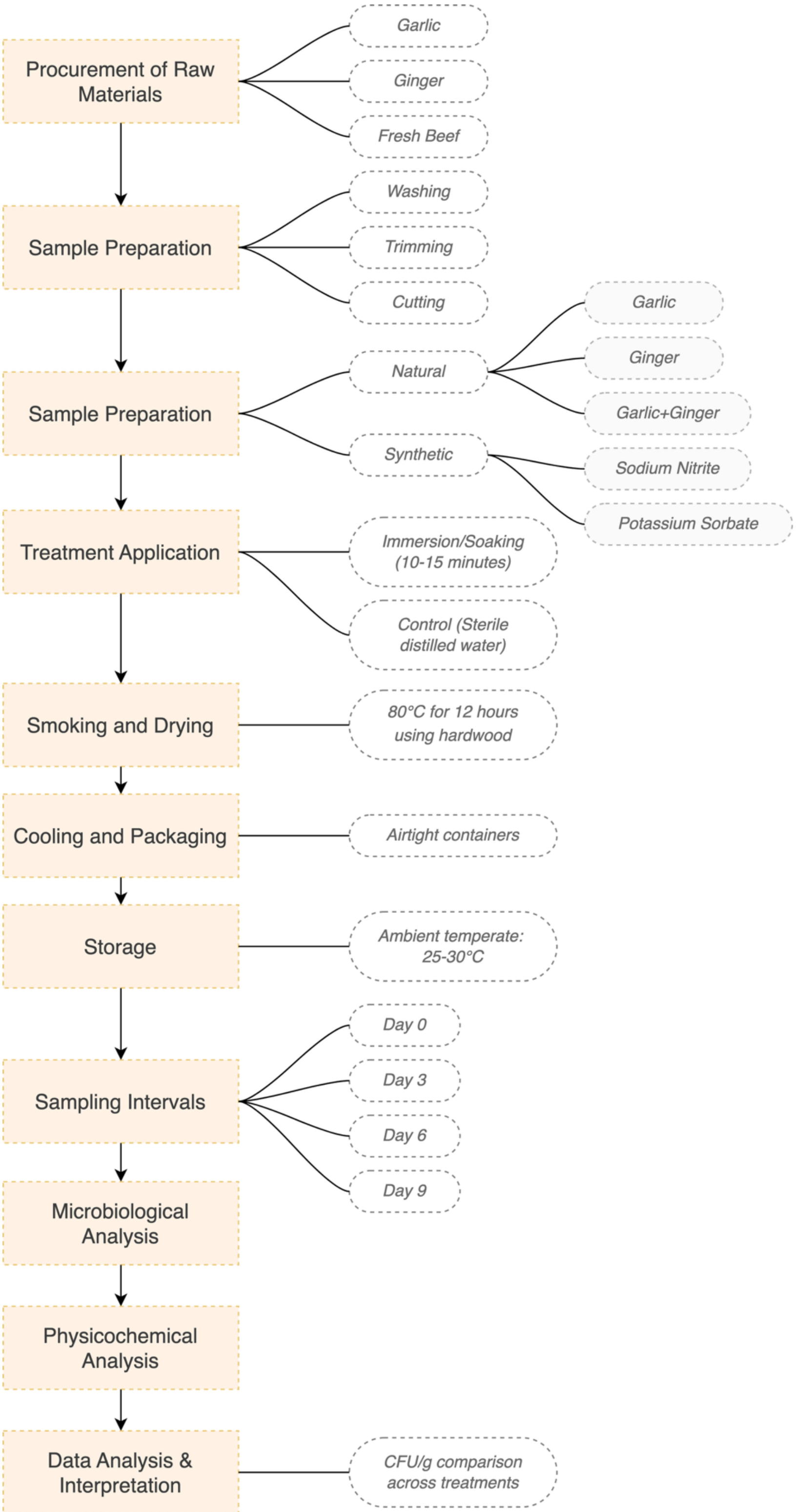

Fig. 1. A flowchart illustrating the experimental stages

2.2. Sample Collection

The natural preservatives, garlic and ginger, will be bought from the Choba market, and the beef was purchased from butchers at the Choba Abattoir in Rivers State, Nigeria.

2.3. Preparation of the Extracts

The outer coats of the garlic were removed, and the cloves were peeled and finely crushed using a kitchen grinder. The quantity of fresh garlic paste required to achieve the treatment concentration (30 g/kg of meat) was measured in a clean bowl. About 150 g of garlic paste was measured into a clean bowl containing 150 mL of clean water. The solution was thoroughly mixed and filtered, and the filtered solution was used to process the meat before smoking. The same procedure was followed to prepare the ginger extract.

2.4. Preparation of the Samples

The combination of the natural preservatives was prepared by weighing 60 g of peeled ginger and 60 g of peeled garlic. Both samples were washed thoroughly, chopped, and blended with 600 mL of distilled water to obtain a 20% (w/v) combined aqueous extract. The mixture was filtered through a clean muslin cloth, and the filtrate (crude extract) was collected and stored in sterile amber bottles at 4°C until use.

For the synthetic preservatives, potassium sorbate and sodium nitrite were each prepared as stock solutions. A 1% (w/v) potassium sorbate stock was obtained by dissolving 10 g of potassium sorbate in 1 L of distilled water, while a 0.1% (w/v) sodium nitrite stock (equivalent to 1 g L^{-1} or 1000 ppm) was prepared by dissolving 1 g of sodium nitrite in 1 L of distilled water. Both solutions were properly labelled and stored in amber bottles at 4°C until required. Working solutions were prepared from these stocks by dilution to the desired concentrations immediately before use. Meat samples were immersed in the respective natural or synthetic preservative solutions for about 10 minutes, drained, and then dried under controlled conditions. Control samples were treated similarly using sterile distilled water. All solutions and equipment used in the preparation process were sterilized to ensure aseptic conditions and prevent contamination.

2.5. Sample Preparation for the Natural Preservatives

The dried meats (beef) were washed with water, trimmed of fat and connective tissues, and cut into small lumps. They were sorted into 12 sets of 5 kg of beef each for drying. The first six sets were divided into three batches of 5 kg each. Each batch of meat was dipped into separate spice juice extracts. The meat samples were dipped into treatments containing a mixture of ginger and garlic extracts, with specimens labelled A for ginger extract, B for garlic extract, and C for the ginger plus garlic extract mixture. The samples were allowed to soak for 15 minutes.

Each batch was demarcated with a wooden rod on the wire gauze placed on top of the smoking kiln and smoked with tropical hardwood. The initial temperature was kept low to prevent surface drying of the meat samples. A temperature of about 80°C was maintained, and the samples were removed after 12 hours of curing. The dried samples were cooled for 30 minutes, packaged in airtight containers, and stored at ambient temperatures of 25°C to 30°C for 9 days.

2.6. Sample Preparation for the Synthetic Preservatives

The remaining six sets of meat samples were dried in pickle infusion solutions containing different concentrations of sodium nitrite and potassium sorbate. A ratio of 500 g of meat to 500 mL of infusion solution was used. The beef was first soaked for 15 minutes and then cooked for 15 minutes. After this, the beef was heat dried. The samples were labeled for those infused with sodium nitrite, some infused with potassium sorbate, and the third one was treated with an infusion of both sodium nitrite and potassium sorbate.

2.7. Storage and Sampling

The samples were packed in airtight containers and stored at ambient temperatures of 25ºC, from where random samples were taken for analysis. Subsequent sampling was carried out at intervals. Twenty-five grams (25 g) of each sample were weighed and homogenized in 225 ml of sterile peptone solution under aseptic conditions. Microbiological analysis was carried out on days 0, 3, 6, and 9 of storage. Tenfold serial dilutions of the dried meat samples were prepared by transferring 1 mL of the stock solution into a test tube containing 9 mL of sterile peptone water solution. The process was repeated for subsequent dilutions to achieve appropriate concentrations for plating.

2.8. Microbiological Analysis

2.8.1. Total Bacteria Count (TBC)

A 0.1 mL aliquot of each dilution was spread onto the surface of pre-poured and dried Plate Count Agar plates. The plates will be incubated at 37°C for 24–48 hours, after which the bacterial colonies will be counted and recorded as colony-forming units per gram (CFU/g) of meat.

2.8.2. Total Fungi Count (TFC)

A 0.1 mL aliquot of each dilution was spread onto the surface of pre-poured and dried potato dextrose agar plates. The plates were incubated at 25°C for 5–7 days, allowing fungal colonies to develop. The colonies were counted and expressed in CFU/g.

2.8.3. Total Staphylococcus Count (TSC)

0.1 mL of the diluted meat homogenate will be spread onto Mannitol Salt Agar plates and incubated at 37°C for 48 hours. Colonies that appear yellow due to mannitol fermentation will be counted separately from non-fermenting colonies and recorded as CFU/g. The presence of high Staphylococcus counts may indicate contamination from handling or inadequate processing.

2.8.4. Total Coliform Count

A 0.1 mL aliquot of each dilution was spread onto the surface of pre-poured and dried MacConkey plates. The plates were incubated at 37°C for 24–48 hours, after which the bacterial colonies will be counted and recorded as colony-forming units per gram (CFU/g) of meat.

2.9. Physicochemical Analysis

The physicochemical analysis includes the following parameters:

2.9.1. Moisture Content

Moisture content was determined by the gravimetric method [21]. The moisture content was calculated as a percentage of moisture loss to the weight of the sample analyzed. Using this procedure, the meat samples are dried at a regulated temperature in an oven until they reach a consistent weight. Using an analytical scale, 5 g of each dried beef sample will be precisely weighed to start the process. To ensure coherence, all samples will be conducted within similar conditions and placed in crucibles that have been subsequently dried and weighed.

After that, the crucibles holding the meat samples will be placed inside a hot air oven that has been preheated to 105°C. To guarantee that all the moisture is gone, the drying process will last for twenty-four hours. Following this time, the crucibles will be taken out with tongs, cooled in a desiccator to stop the reabsorption of moisture, and then weighed again. Every sample's ultimate weight will be noted.
The formula below was used in the calculation of:

$$\% \, Moisture \, Content \; = 100 \, x \, \frac{W_1 - W_2}{W_1} \quad (1)$$

W_1= Initial weight
W_2= Final weight
All samples kept in various treatments will go through this procedure, including those preserved with artificial preservatives like potassium sorbate and sodium nitrite as well as natural ones such extracts of ginger and garlic. To evaluate trends in moisture retention over time, the study test and reading will be taken at day 0 and day 9.

2.9.2. Total Volatile Base Nitrogen (TVB-N)

A 10 g sample of meat will be homogenized with distilled water and subjected to steam distillation. The distillate will be collected in a flask containing boric acid, and the liberated ammonia will be quantified by titration with a standardized acid solution. The final TVB-N value was expressed in milligrams of nitrogen per 100 grams of meat (mg N/100g). This analysis will be performed at regular intervals during the storage period to monitor changes over time and determine which preservation method best prevents protein degradation [22].

2.9.3. Statistical Analysis

Data were analyzed using two-way ANOVA to assess the effects of preservative treatment and storage time on microbial and physicochemical parameters. Mean separation was performed using Tukey's HSD test at $p < 0.05$. Results were expressed as mean ± standard deviation, and analyses were conducted using SPSS at a 95% confidence level.

3. RESULTS AND DISCUSSION

Meat is a highly nutritious commodity but is also highly perishable due to its rich protein and moisture composition. Without adequate preservation, dried meat is prone to quality deterioration, food safety risks, and economic losses. Microbial proliferation during storage is one of the major factors influencing the quality and shelf life of dried beef. The application of preservatives is therefore essential to retard microbial growth and maintain product safety. Both sodium nitrite and potassium sorbate are widely used at low inclusion levels to achieve effective antimicrobial activity while adhering to regulatory safety limits.

In this study, microbial counts demonstrated significant differences ($p \leq 0.05$) among treatments. The total viable bacterial count (**Fig. 2**) increased progressively throughout storage across all samples; however, the rate of increase varied depending on preservative treatment. The untreated control exhibited the highest microbial proliferation, whereas preserved samples showed slower increases. Samples treated with synthetic preservatives (M + PS, M + SN, and M + PS + SN) showed moderately elevated initial counts (3.77–3.97 log CFU/g) at day 0 but demonstrated restrained increases by day 9 (5.6–6.0 log CFU/g), consistent with the inhibitory properties of nitrite and sorbate.

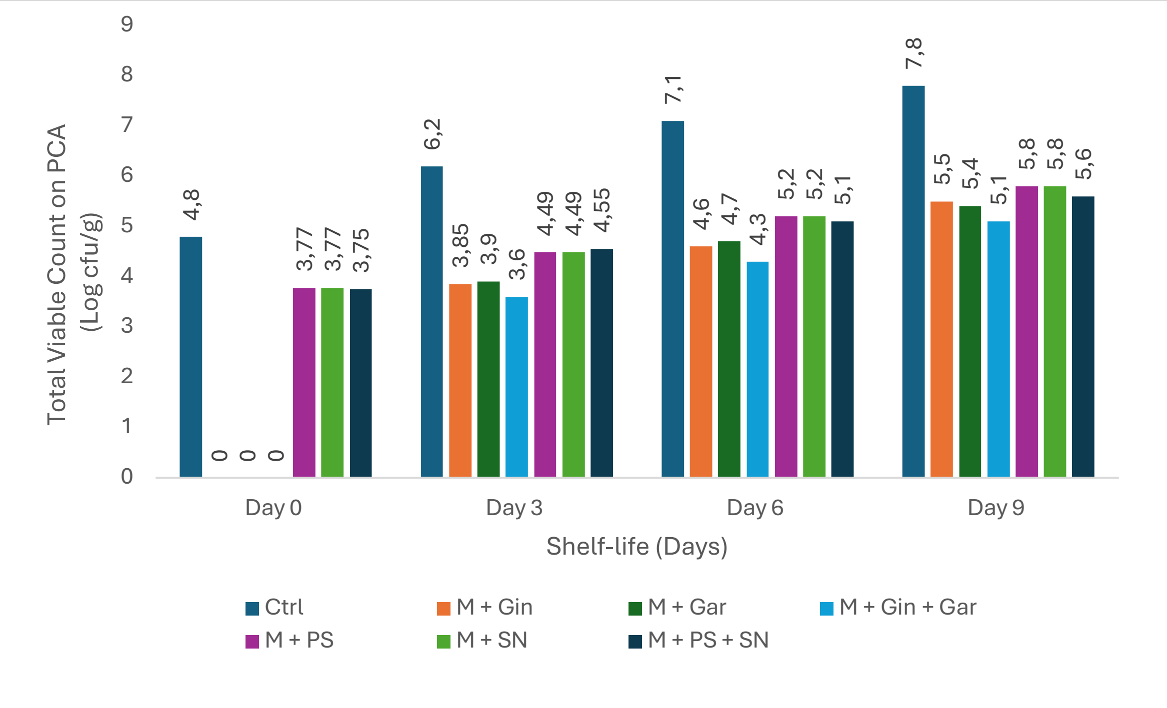

Fig. 2. Total viable count of the different dried beef samples

Natural preservative treatments (ginger, garlic, and their combination) showed no detectable microbial counts at day 0 and increased moderately by day 9. This pattern aligns with previous reports indicating that ginger and garlic contain potent antimicrobial phytochemicals such as gingerols, shogaols, and allicin that suppress microbial growth and extend shelf life. Similar findings were reported by Sallam et al. [10], who observed strong antimicrobial effects of garlic (fresh, powder, and oil forms) on raw chicken sausage. Likewise, Idris et al. [23], Tagoe et al. [24], Kumolu-Johnson and Ndimele [25], Kutte [26], Shaukat [27], and El-Saadony et al. [28] documented significant antibacterial effects of ginger and garlic extract in various food matrices. Joe et al. [29] further demonstrated that garlic extract exerted superior antibacterial activity against several pathogens at concentrations ranging from 1000 to 2000 ppm. Thongson et al. [30] also reported inhibitory effects of ginger extracts against Listeria monocytogenes and Salmonella Typhimurium DT104. Comparative studies on spices by Indu et al. [31] revealed the antimicrobial potential of ginger against selected Escherichia coli serogroups, supporting its role as an effective natural preservative.

The antimicrobial roles of nitrites and sorbates in cured or treated meats are well established and explain the moderated bacterial growth observed in this study. Potassium sorbate dips have been shown to significantly extend the shelf life of fresh chicken cuts [32], [33]. Similarly, Apata et al. [34] and Terrell et al. [35] noted that beef franks formulated with 50 ppm nitrite and 0.26% potassium sorbate were comparable in sensory quality to those containing 156 ppm nitrite. Additional findings by González and Díez [36] confirmed the effectiveness of nitrite (50–150 ppm) in reducing Enterobacteriaceae in cured meat products.

Although natural preservatives have gained attention as safer alternatives to synthetic additives due to increasing consumer health concerns, this study demonstrates that both natural and synthetic preservatives effectively inhibited microbial growth. However, combinations of preservatives, both natural and synthetic, tended to provide superior antimicrobial effects. This synergistic activity has been reported by Kimani et al. [37], Aziz et al. [38], and Mediani et al. [39], who observed enhanced microbial inhibition when preservatives were combined.

The total coliform count (**Fig. 3**) remained lower in treated samples compared with the untreated control throughout storage. Synthetic preservatives maintained steadily increasing but moderate coliform counts by day 9 (5.6–6.1 log CFU/g). Natural treatments showed lower coliform growth during intermediate storage (days 3–6), although counts increased by day 9. These trends reflect the selective antimicrobial activity of sorbates and nitrites against Gram-negative bacteria, while phytochemicals in ginger and garlic also exert inhibitory effects in a concentration-dependent manner. These findings align with earlier studies demonstrating that multiple preservation hurdles are more effective than single preservation methods in suppressing Enterobacteriaceae [40], [41].

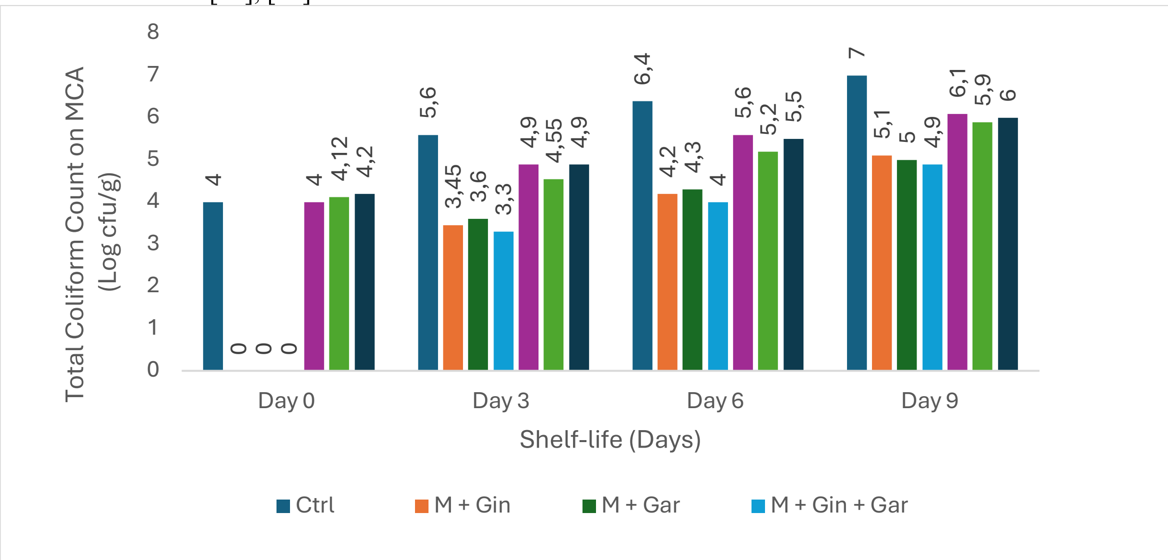

Fig. 3. Total coliform count of the different dried beef samples

Staphylococcal counts (**Fig. 4**) showed initial populations of 3.30–3.52 log CFU/g in synthetic preservative treatments, while natural preservative samples showed no detectable counts at day 0 (except the ginger–garlic combination). Although counts increased during storage, growth was slower in preserved samples than in the untreated control. Nitrite inhibits Gram-positive bacteria such as Staphylococcus spp., while sorbate disrupts cellular enzyme systems. Natural extracts, particularly when combined, also demonstrated inhibitory effects, though efficacy varied with formulation and meat composition.

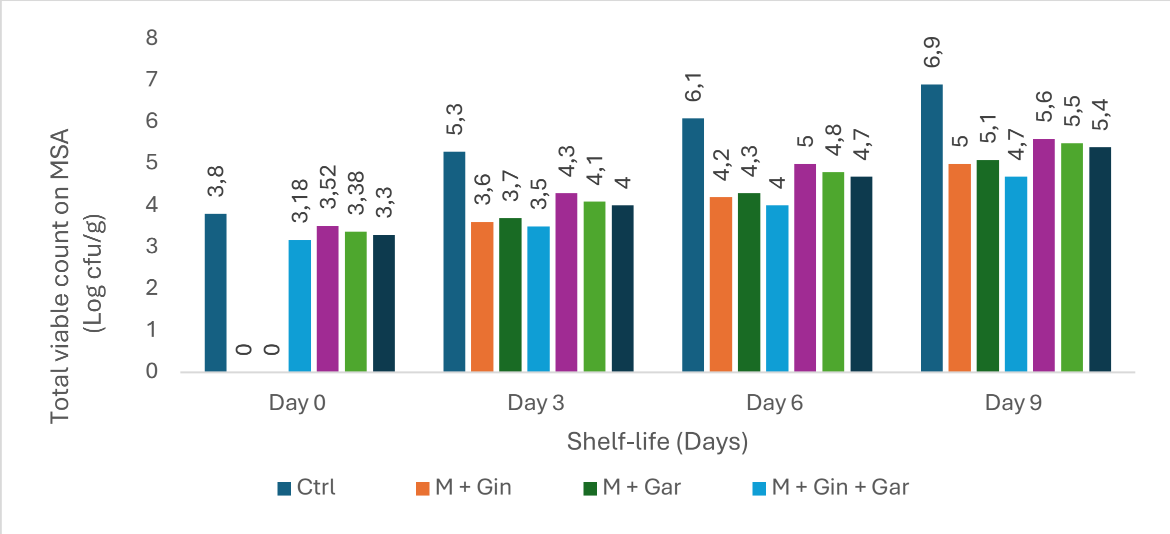

Fig. 4. Staphylococcal count of the different dried beef samples

Fungal counts (**Fig. 5**) increased across all treatments during storage; however, potassium sorbate showed the strongest antifungal activity. Ginger and garlic treatments were more frequently associated with yeast growth, while moulds such as Aspergillus niger appeared across multiple samples. These findings agree with reports indicating that plant extracts may selectively inhibit moulds while permitting limited yeast growth [42], [43]. Overall, preservatives slowed fungal proliferation but did not completely prevent growth, emphasizing the importance of combined preservation strategies.

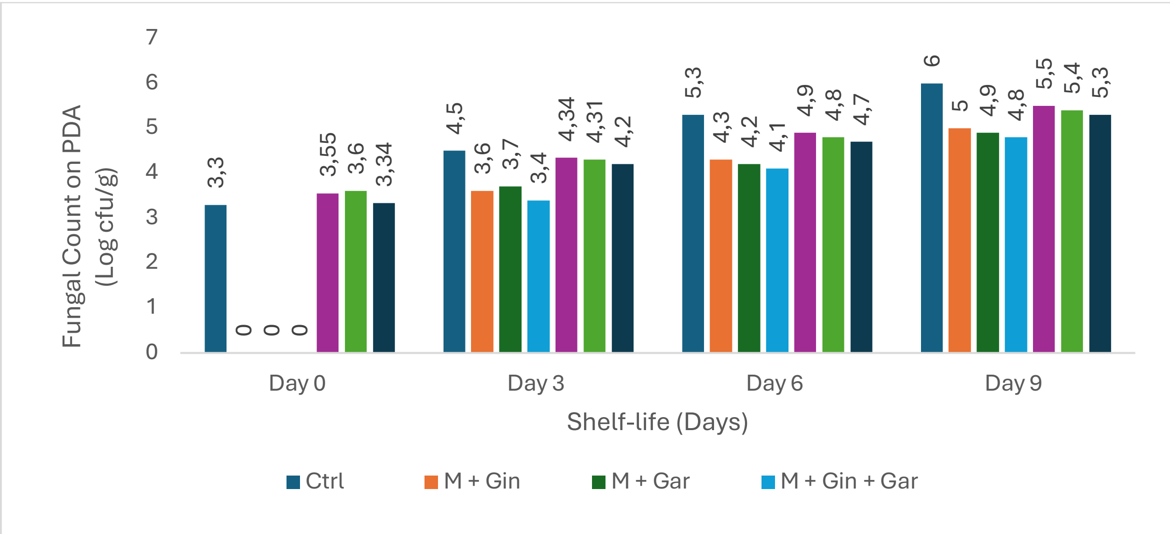

Fig. 5. Fungal count of the different dried beef samples

Overall, both natural (ginger, garlic, and their combination) and synthetic (potassium sorbate, sodium nitrite, and their combination) preservatives significantly reduced microbial growth during nine days of storage. Synthetic preservatives exhibited more consistent inhibition, while combined natural extracts approached comparable efficacy. These findings support hurdle technology approaches combining dehydration, hygienic handling, and antimicrobial treatments to enhance meat safety and shelf life [27], [28], [44][45][46].

Bacterial analysis (**Fig. 6**) revealed four dominant genera: Bacillus spp., Pseudomonas aeruginosa, Staphylococcus aureus, and Escherichia coli. Bacillus spp. was most prevalent due to their spore-forming ability and resistance to desiccation, consistent with previous reports [47], [48]. The occurrence of Pseudomonas aeruginosa suggests possible post-processing contamination, as these organisms are associated with moisture-dependent spoilage [49][50][51]. The detection of Staphylococcus aureus indicates handling contamination, while low E. coli prevalence suggests relatively good hygiene [40].

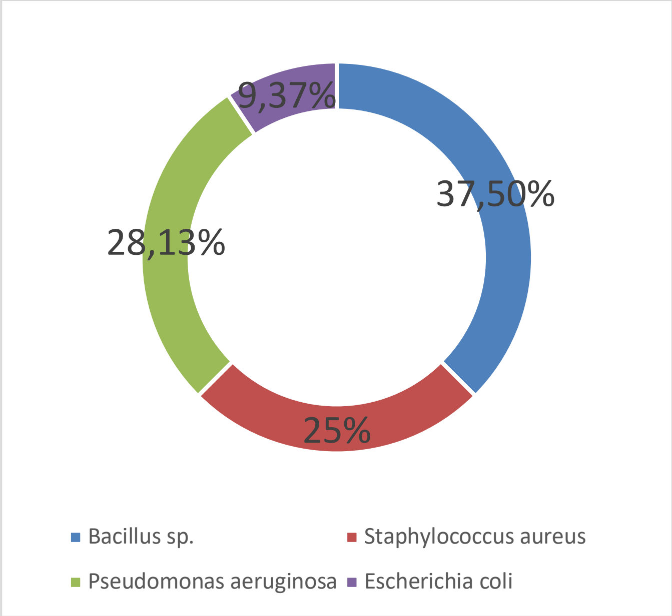

Fig. 6. Percentage frequency occurrence of bacteria found in the dried beef samples

Fungal isolates (**Fig. 7**) were dominated by Aspergillus niger, followed by Saccharomyces cerevisiae and Candida spp. The dominance of A niger reflects its environmental resilience and ability to grow at low water activity [42], [43]. Limited yeast occurrence aligns with reports that some natural extracts suppress molds while allowing osmophilic yeasts to persist [42]. Chemical preservatives showed stronger antifungal effects [45].

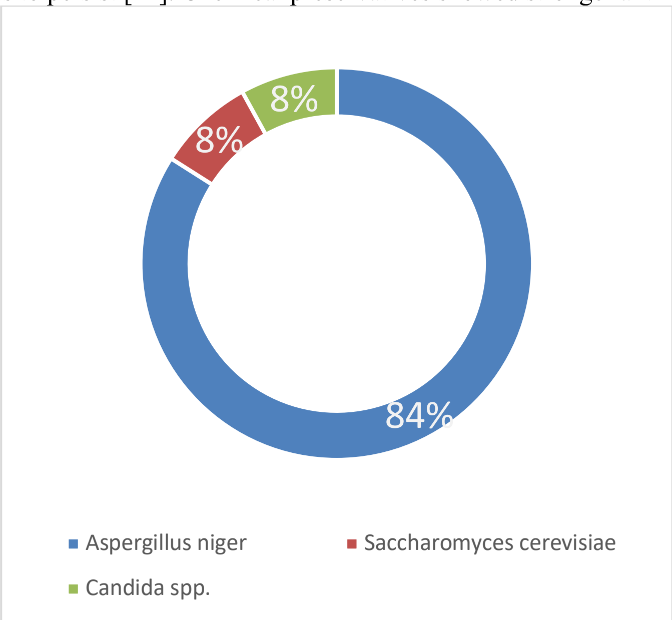

Fig. 7. Percentage frequency occurrence of fungi found in the dried beef samples

Gram staining and biochemical tests confirmed the presence of Bacillus subtilis, Staphylococcus aureus, Pseudomonas aeruginosa, and Escherichia coli, consistent with earlier findings on dried meat spoilage [52][53][54]. Synthetic preservatives effectively reduced microbial diversity, particularly when combined, supporting previous studies [15], [55][56][57].

As seen in Table 1, moisture content decreased progressively across all treatments, with the control retaining the highest moisture. Synthetic preservative combinations exhibited the lowest moisture levels, indicating reduced water activity and enhanced stability. Similar trends have been reported in dried meat systems [58]. Reduced moisture limited microbial activity and protein degradation, as reflected in TVB-N values as seen in Table 2 [59][60][61].

Table 1. Effect of Natural and Artificial Preservatives on the Moisture Content (%) of Dried Beef Meat during Storage

Samples	Day 0	Day 3	Day 6	Day 9
Ctrl	32.50 ± 0.15[a]	31.20 ± 0.10[a]	29.85 ± 0.20[a]	28.10 ± 0.25[a]
M + Gin	31.80 ± 0.20[ab]	30.45 ± 0.15[ab]	29.10 ± 0.10[ab]	28.00 ± 0.15[ab]
M + Gar	31.60 ± 0.18[b]	30.30 ± 0.12[b]	28.95 ± 0.20[b]	27.90 ± 0.18[b]
M + Gin + Gar	31.40 ± 0.22[bc]	30.10 ± 0.18[bc]	28.80 ± 0.15[bc]	27.75 ± 0.20[bc]
M + PS	30.90 ± 0.15[c]	29.70 ± 0.12[c]	28.50 ± 0.10[c]	27.40 ± 0.15[c]
M + SN	30.60 ± 0.10[cd]	29.50 ± 0.15[cd]	28.30 ± 0.18[cd]	27.20 ± 0.12[cd]
M + PS + SN	30.40 ± 0.12[d]	29.30 ± 0.10[d]	28.10 ± 0.15[d]	27.00 ± 0.18[d]

Values are means of triplicates ± S.D. Data with the same superscripts within the same column are not significantly different ($p < 0.05$).

Table 2. Effect of Natural and Artificial Preservatives on Total Volatile Base Nitrogen (TVB-N, mg N/100 g) of Dried Beef Meat during Storage.

Samples	Day 0	Day 3	Day 6	Day 9
Ctrl	7.25 ± 0.10[a]	12.80 ± 0.15[a]	18.90 ± 0.20[a]	24.60 ± 0.25[a]
M + Gin	7.10 ± 0.12[ab]	10.45 ± 0.10[ab]	14.60 ± 0.15[ab]	18.30 ± 0.18[ab]
M + Gar	7.00 ± 0.10[b]	10.20 ± 0.15[b]	14.30 ± 0.12[b]	17.90 ± 0.15[b]
M + Gin + Gar	6.95 ± 0.10[bc]	9.95 ± 0.12[bc]	13.90 ± 0.10[bc]	17.40 ± 0.18[bc]
M + PS	6.80 ± 0.15[c]	9.10 ± 0.10[c]	12.50 ± 0.15[c]	15.80 ± 0.12[c]
M + SN	6.75 ± 0.12[cd]	8.90 ± 0.10[cd]	12.20 ± 0.10[cd]	15.40 ± 0.15[cd]
M + PS + SN	6.70 ± 0.10[d]	8.70 ± 0.12[d]	11.90 ± 0.10[d]	15.00 ± 0.10[d]

Values are means of triplicates ± S.D. Data with the same superscripts within the same column are not significantly different ($p < 0.05$).

Overall, artificial preservatives particularly potassium sorbate and sodium nitrite in combination provided superior microbial and biochemical stability. Nevertheless, natural preservatives such as ginger and garlic demonstrated meaningful inhibitory effects and remain valuable as safer alternatives or complementary agents in optimized preservation strategies.

4. CONCLUSION

This study demonstrated that both synthetic preservatives (potassium sorbate and sodium nitrite) and natural extracts (ginger and garlic) significantly inhibited microbial growth, with synthetic preservatives providing more consistent control while combined natural extracts approached comparable efficacy. The findings highlight the potential of combining artificial and natural preservatives to achieve synergistic antimicrobial effects, reduce moisture activity, and maintain biochemical stability. Therefore, integrated preservation strategies that combine dehydration, hygienic handling, and complementary use of synthetic and natural preservatives offer an effective approach to extending dried meat shelf life while addressing consumer safety and health concerns.

List of Abbreviations

List of Abbreviations	Abbreviations Meaning
M	Meat
PS	Potassium sorbate
SN	Sodium nitrite
Gin	Ginger
Gar.	Garlic
Ctrl	Control
TVB-N	Total Volatile Base Nitrogen

Declaration Author Contributions

Omorordion Nnenna J.: Conceptualization, Methodology, Supervision, Writing – Review & Editing, Validation; Wami Princess C.: Investigation, Formal analysis, Data curation, Writing – Original Draft, Writing – Review & Editing, Validation.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request. All relevant data generated or analyzed during this study are included within this article and its supplementary materials.

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