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# Effectiveness of Herbs and Spices as Natural Antioxidants in Preserving Tuna (*Thunnus* sp.) and Nile Tilapia (*Oreochromis niloticus*): Enhancing Microbial Safety and Shelf Life

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**Abstract:** Fish is a protein-rich and highly nutritious food. Natural herbs and spices contain antioxidant and antibacterial compounds that may enhance microbiological safety and extend shelf life. This study aimed to evaluate natural herb and spice-based processing methods for preserving tuna (*Thunnus* sp.) and nile tilapia (*Oreochromis niloticus*), along with the effectiveness of specific spices in reducing oxidation and microbiological contamination. Two marination approaches were applied, using both uncooked and cooked spice mixtures. Three recipes were prepared, recipe 1: garlic, pepper, salt, turmeric, coriander, lemongrass, recipe 2: garlic, pepper, salt, rosemary, oregano, thyme, lemon and recipe 3: garlic, pepper, salt, coriander, lemongrass, chili pepper, curry leaves, asam sunti. Unmarinated fish served as the control. All samples were vacuum-packed in retort pouches and stored at 1-4 °C for 1 h, 3, 5, and 7 days. Microbial analysis included total aerobic bacteria, *Escherichia coli*, *Coliforms*, *Salmonella* spp., *Shigella* spp., yeast and mold. Marination with herbs and spices significantly reduced microbial contamination compared to the control. The combined use of herbs and spices effectively improved microbial safety and extended the shelf life of tuna and nile tilapia. These findings support the application of herbs and spices as natural preservation agents in fish products, offering a safer and more sustainable alternative in fish preservation.

**Keywords:** Antioxidant; Herbs; Nile; Preservation; Spices; Tuna

## Introduction

Fish is one of the most important sources of animal protein and essential micronutrients for human health. It contains high-quality protein, long-chain omega-3 fatty acids, vitamin A, vitamin D, vitamin B12, iodine, iron, selenium, and zinc, all of which are crucial for growth, development, and disease prevention (Byrd et al., 2021). Increasing fish consumption is associated with reduced risks of micronutrient deficiencies, which remain widespread, particularly among vulnerable populations such as preschool children and women of reproductive age (Golden et al., 2021; Stevens et al., 2022). Tuna (*Thunnus* sp.) is one of the most widely consumed marine fish due to its favorable nutritional composition and sensory properties (Chamorro et al., 2024). Nile tilapia (*Oreochromis niloticus*) is a major freshwater aquaculture product in Southeast Asia, including Indonesia, where it plays a critical role in household food security and local economies (Utomo et al., 2025). Therefore, promoting higher fish consumption is an effective strategy to improve dietary quality and address protein-energy malnutrition.

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Despite these benefits, fish is highly perishable. Spoilage occurs rapidly after harvest due to biochemical changes, enzymatic activity, microbial contamination, and oxidative reactions in lipids and proteins. It is estimated that up to 30% of landed fish worldwide is lost before reaching consumers, with microbial contamination being the leading cause of post-harvest loss (Ghaly et al., 2010). Common pathogenic microorganisms associated with fish include *Salmonella* spp. (Porto et al., 2023; Reis et al., 2024), *Listeria monocytogenes* (Lambrechts & Rip, 2024), *Vibrio* spp. (Abdalla et al., 2022), and parasites such as *Anisakis* (Setyobudi et al., 2023). These organisms not only reduce fish quality but also pose risks of foodborne illness. For producers and retailers, spoilage and contamination represent major economic losses due to reduced shelf life and marketability.

Conventional preservation strategies, such as refrigeration, freezing, and vacuum packaging, can slow microbial growth and oxidation, but they are often insufficient for ensuring long shelf life without additional interventions. In response, there has been growing interest in natural preservation methods. Herbs and spices are particularly attractive because they contain bioactive compounds with antimicrobial, antioxidant, and anti-inflammatory properties (POP et al., 2019). Their phenolic constituents, including flavonoids, terpenes, and essential oils, play a key role in inhibiting microbial growth and preventing oxidative damage in food matrices (Tymczewska et al., 2023; Widhianata et al., 2022). Spices such as garlic, turmeric, rosemary, oregano, thyme, and cumin have shown promising preservative effects in meat and fish systems, but most studies have investigated them individually or in simple mixtures (Jiménez-Ruíz et al., 2023; Olivas-Méndez et al., 2022).

Marination is one of the simplest and most traditional methods of improving the safety and quality of fish products. By immersing fish in liquid solutions containing spices, acids, and enzymes, marination can reduce microbial load, improve flavor, and enhance texture (Lopes et al., 2022). The process facilitates the penetration of antimicrobial and antioxidant compounds into fish tissue through osmosis, thereby creating a less favorable environment for spoilage organisms (Gómez et al., 2020). Studies have demonstrated that marination not only enhances sensory properties but also contributes to prolonged storage stability. When combined with packaging innovations such as vacuum sealing or retort pouches, marination offers a promising natural approach to extending the shelf life of fish and seafood products (Dixon et al., 2020; Mafe et al., 2024).

Previous studies have generally emphasized a limited scope, often focusing on single spices or on one type of fish, either marine or freshwater. In addition, little comparative work has been done to evaluate the influence of cooked versus uncooked marination on microbial reduction and oxidative stability during storage. To address these limitations, this study applies combinations of herbs and spices to both tuna and nile tilapia, representing marine and freshwater species, and compares two marination methods under cold storage conditions.

To achieve these objectives, three spice formulations reflecting different culinary traditions were applied: recipe 1 contain mixture of garlic, pepper, salt, turmeric, coriander, and lemongrass; recipe 2 contain of garlic, pepper, salt, rosemary, oregano, thyme, and lemon; and recipe 3 contain garlic, pepper, salt, coriander, lemongrass, chili pepper, curry leaves, and asam sunti. Both uncooked and cooked marination methods were compared, while unmarinated fish served as the control. The evaluation focused on microbial safety, including aerobic bacteria, *Escherichia coli*, *Coliforms*, *Salmonella*, *Shigella*, yeast, and mold, as well as physicochemical indicators such as pH and oxidation-reduction potential (ORP), to determine the effectiveness of each treatment in extending fish shelf life. Therefore, this study aimed to investigate the effectiveness of herbs and spices as natural antioxidants in preserving tuna and Nile tilapia. The focus was placed on their ability to reduce microbial contamination, inhibit oxidation, and extend the shelf life of fish products stored under refrigeration.

Therefore, this study aimed to investigate the effectiveness of herbs and spices as natural antioxidants in preserving tuna and Nile tilapia. The focus was placed on their ability to reduce microbial contamination, inhibit oxidation, and extend the shelf life of fish products stored under refrigeration. Unlike previous studies focusing on a single fish species or individual spices, this research evaluates the preservative efficacy of complex spice blends on two economically and nutritionally important fish, tuna (marine) and Nile tilapia (freshwater), providing insights that are widely applicable to diverse aquaculture and culinary contexts, generating robust comparative data on their effectiveness in controlling microbial growth and preventing oxidation.

This research is crucial for advancing sustainable, health-oriented fish preservation strategies using natural bioactive compounds from herbs and spices. This research also to advance sustainable, health-promoting fish preservation methods that harness the functional properties of herbs and spices, with potential benefits for public health, household nutrition, and local economies.

## Method

*Time and Place*

The research was conducted from June to September 2025 at the Integrated Science Laboratory of Universitas Kristen Cipta Wacana.

*Food Materials*

Two fish species were selected to represent different aquatic environments: tuna (*Thunnus* sp.) as a marine fish and nile tilapia (*Oreochromis niloticus*) as a freshwater fish. The marination solutions were prepared using a variety of herbs and spices with known antioxidant and antimicrobial properties, including garlic, pepper, turmeric, cumin, lemongrass, coriander, oregano, rosemary, thyme, lemon, chili pepper, curry leaves, and *asam sunti*.

*Marination Treatment*

Marination was carried out using two approaches: uncooked spice mixtures and cooked spice mixtures. Three spice formulations were designed include recipe 1: garlic, pepper, salt, turmeric, coriander, cumin and lemongrass; recipe 2: garlic, pepper, salt, rosemary, oregano, thyme, and lemon; recipe 3: garlic, pepper, salt, coriander, lemongrass, chili pepper, cumin, curry leaves, and *asam sunti*. Unmarinated fish served as the control.

*Packaging and Storages*

Following marination, both treated and control samples were vacuum-sealed in heat-resistant retort pouches and stored at refrigeration temperature (1-4°C). Shelf life was assessed across four storage periods: 1 h (D0), 3 days (D3), 5 days (D5), and 7 days (D7).

*Observation*

*Physicochemical Analysis*

Physicochemical properties were determined by measuring pH and oxidation-reduction potential (ORP) using a digital meter Constant WT501. All measurements were performed in triplicate.

*Microbiological Analysis*

Microbiological quality was assessed by enumerating total aerobic bacteria, *Escherichia coli*, *Coliforms*, *Salmonella* spp., *Shigella* spp., yeast, and mold. Selective Petrifilm plates were employed for microbial colony enumeration, with specific media as follows: total aerobic bacteria using Neogen® Petrifilm® Aerobic Count (AC) Plates, total *E. coli* and Coliforms using Neogen® Petrifilm® *E. coli*/Coliform Count (EC) Plates, total *Salmonella* spp. and *Shigella* spp. using Salmonella–Shigella Agar (SSA) differential medium (HiMedia®), total yeast and mold using Neogen® Petrifilm® Rapid Yeast and Mold Count (RYM) Plates.

*Sample Preparation*

Samples were prepared by homogenizing 25 g of fish tissue with 225 mL of sterile aquadest, followed by serial dilutions up to $10^{-5}$. From each dilution, 1 mL aliquots were inoculated onto AC, EC, and RYM Petrifilm plates, as well as SSA agar. All procedures were performed in triplicate.

*Inoculation*

Microbial inoculation onto Petrifilm plates was carried out using the drop-and-spread method. The top film was lifted to expose the dehydrated culture medium, followed by dispensing 1 mL of the diluted sample onto the center of the bottom film. The top film was then gently rolled down and the sample evenly spread using a Petrifilm spreader.

*Incubation*

The inoculated plates were incubated under the following conditions: AC Petrifilm: 32–37 °C for 48 h, EC Petrifilm: 35 ± 1 °C for 24 ± 2 h for Coliform enumeration, with an additional 24 ± 2 h (total 48 h) at the same temperature for *E. coli*, RYM Petrifilm: 20–25 °C (room temperature) for 72 h, SSA Agar: 35–37 °C for 24 h for detection of *Salmonella* spp. and *Shigella* spp. All incubations were performed with plates placed horizontally, clear side up.

*Enumeration (Counting)*

Colony counts were recorded manually according to the manufacturer’s guidelines: AC Petrifilm with red colonies (aerobic bacteria, including lactic acid bacteria), EC Petrifilm with *E. coli* colonies appeared as blue to red–blue colonies with gas, *Coliforms* as red or blue colonies associated with gas, blue colonies without gas were not considered *E. coli*. SSA: *Salmonella* colonies appeared colorless with black centers. *Shigella* colonies appeared colorless. RYM Petrifilm with yeast colonies appeared as small, blue–green colonies with well-defined edges and no foci, while mold colonies were larger, variably colored, with diffuse edges and central foci.

*Statistical Analysis*

Data were analyzed using R software. Analysis of variance (ANOVA) was performed to evaluate the effects of treatment and storage duration. Tukey’s Honestly Significant Difference (HSD) post-hoc test and descriptive statistics were used to compare unmarinated and marinated tuna and nile tilapia across different spice compositions and storage times.

## Result and Discussion

Our study examined the effects of marination using a combination of 13 natural spices, including garlic, pepper, turmeric, cumin, lemongrass, coriander, oregano, rosemary, thyme, lemon, chili pepper, curry leaves, and *asam sunti*. These spices are known for their antimicrobial and antioxidant properties, and their combined use not only enhanced the flavor profile but also improved preservation effectiveness. The appropriate composition of these herbs and spices played a critical role in physicochemical properties and inhibiting microbial growth during storage.

### *The Effectiveness of Herbs and Spices on the Physicochemical Properties of Fish*

The result indicated that marinated tuna and nile tilapia, regardless of the cooking method, exhibited lower pH values than their respective control samples (Figure 1). Lower pH levels are associated with slower microbial activity and protein degradation, which contributes to prolonged shelf-life (Fitri et al., 2022; Mafe et al., 2024). These findings suggest that the combined use of refrigeration and spice-based marination can significantly improve the microbiological and physicochemical stability of fish during cold storage.

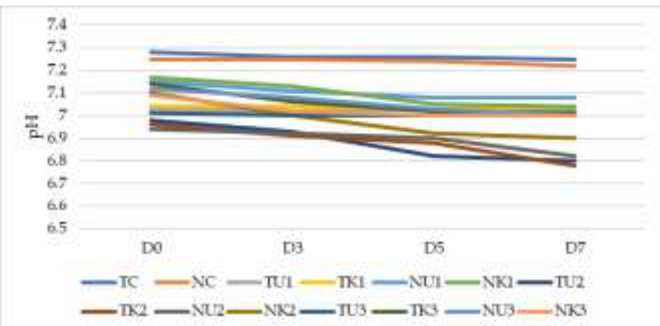


**Figure 1**. pH changes in unmarinated and marinated fish samples during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

As shown in Figure 1, all samples exhibited a gradual decline in pH over the 7-day storage period, which is consistent with microbial and enzymatic activities leading to acid production during cold storage. However, the decrease in pH was significantly more pronounced in the control samples (TC and NC), with final pH values dropping below 6.9 on day 7. In contrast, marinated samples, particularly those prepared using recipe 3 (e.g., TU3, NU3, TK3, and NK3), maintained relatively stable pH levels throughout the storage period.

These results suggest that herbal marinades were effective in suppressing microbial activity, thereby slowing acid production and maintaining pH stability. This is in line with previous findings that spices such as turmeric, garlic, lemongrass, and curry leaves can inhibit microbial growth and preserve fish quality (Mafe et al., 2024; Parmar et al., 2024a). Furthermore, both cooked and uncooked versions of the marinades showed similar trends, indicating that the antimicrobial properties of the herbs remained effective regardless of cooking treatment. The stabilization of pH in marinated fish supports the hypothesis that natural spices can contribute not only to flavor enhancement but also to functional preservation effects. Maintaining a higher and more stable pH is essential in limiting spoilage and extending shelf-life during refrigerated storage.

The pH of live fish immediately after slaughter generally remains close to 7.0 but tends to decrease post-mortem due to the accumulation of lactic acid from glycogen breakdown during rigor mortis (Jiménez-Ruíz et al., 2023). In this study, both tuna and tilapia fillets—marinated and control—showed a gradual decline in pH during 7 days of refrigerated storage (Figure 1). However, a consistent trend was observed where marinated samples had significantly lower pH values ($p < 0.05$) than the unmarinated controls throughout storage. For example, on day 7, the pH values of control samples (TC and NC) remained around 7.2–7.3, while most marinated samples decreased to below 7.0.

Conversely, the slight pH increase in control fillets may be due to the production of volatile basic nitrogen compounds like ammonia and biogenic amines (e.g., putrescine, histamine, cadaverine), which are known by products of microbial degradation (Liu et al., 2010). Garlic-marinated tilapia, for instance, maintained a pH of 7.04 ± 0.143, compared to 7.24 ± 0.028 in the control group (Jiménez-Ruíz et al., 2023).

In addition to pH, the oxidation-reduction potential (ORP) of the samples was measured as an indicator of oxidative stability (Figure 2). ORP is affected by redox reactions and reflects the tendency of a system to gain or lose electrons (Banhidi, 2021). At the beginning of storage (D0), unmarinated control samples (TC and NC) had higher ORP values (~140–145 mV) compared to marinated fish (~120–125 mV). Over time, ORP in control samples increased, reaching values above 150 mV by day 7. In contrast, the ORP of marinated samples remained relatively stable or slightly decreased, maintaining values between 115–125 mV (Figure 2).

This lower and more stable ORP in marinated fillets indicates a less oxidative environment, likely due to the antioxidant properties of certain herbs and spices such as rosemary, garlic, thyme, turmeric, and oregano

(Bieżanowska-Kopeć & Piatkowska, 2022; Parmar et al., 2024a). These spices contain compounds like carnosic acid and rosmarinicacid, which scavenge free radicals and inhibit lipid peroxidation. In fact, rosemary alone has been shown to reduce oxidation parameters by more than 50% compared to control samples (Parmar et al., 2024a).

The simultaneous reduction in pH and ORP in marinated fish indicates that natural spice-based marinades can effectively modify the physicochemical environment to inhibit microbial and oxidative spoilage. This preservation strategy not only extends shelf-life but also maintains product safety and sensory quality. These findings support the integration of natural marinades as a green, health-conscious alternative to synthetic preservatives in fish preservation.

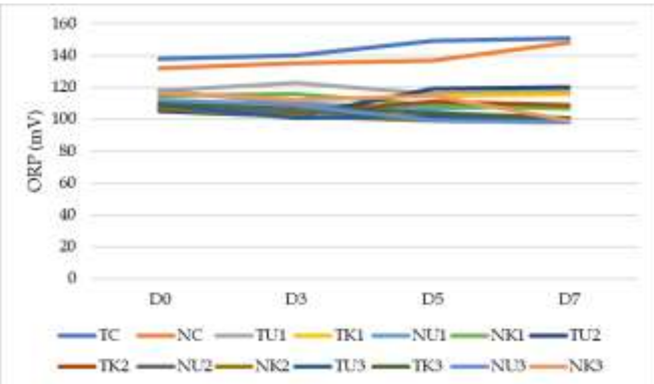


**Figure 2**. Oxidation-Reduction Potential (ORP) in unmarinated and marinated fish during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

*The Effectiveness of Herbs and Spices on the Microbial Growth of Fish*

Furthermore, microbial contamination remains a major factor in fish spoilage and public health risk. High water content in fish provides an ideal medium for microbial proliferation, especially under suboptimal storage conditions (Novak Babič et al., 2020). Microbial growth can be monitored using total viable count (TVC), total coliforms, and fecal coliforms through standard plate count and MPN methods (Sanjee & Karim, 2016). The marination process, especially when combined with cold storage, offers a synergistic approach by creating unfavorable conditions for microbial activity, as shown by the physicochemical parameters.

The results showed that fish samples coated with natural herb-based marinades exhibited significantly reduced microbial counts during seven days of cold storage (1-4°C) compared to the unmarinated control. This finding aligns with previous studies reporting that refrigeration effectively reduces microbial growth in perishable food items (Amit et al., 2017). In addition, the antioxidant properties of the coating slowed lipid oxidation, which is a major contributor to quality deterioriation in fish meat (Kurek et al., 2024). Herbs such as rosemary and thyme have demonstrated both antioxidative and antimicrobial activities, which may explain the extended shelf-life observed in this study (Parmar et al., 2024b). The combination of refrigeration and herbal marinades thus appears to be a promising method to preserve fish quality and safety (Indio et al., 2024).

The effectiveness of herbs and spices as natural ingredients in the preservation period of fish can be determined by testing the microbial content in packaged and stored fish samples using the parameters including total number of aerobic bacteria, total number of *Escherichia coli* and *Coliform,* total number of *Salmonella* sp. and *Shigella,* and total number of yeast and mold. Total bacteria in fish preservation show the overall microbial load, with higher counts indicating poorer hygiene and shorter shelf-life, while lower counts suggest effective preservation methods that inhibit microbial growth. These counts, measured in Colony Forming Units (CFU) per gram or mililiter (Eden, 2014). The different between unmarinated and marinated fish at different storage times can be seen in Figure 1.

Statistical analysis using two-way ANOVA confirmed that marination treatment with three different herb-spice recipes, storage time, and their interaction significantly influenced counts of aerobic bacteria, coliforms, E. coli, Salmonella, Shigella, yeast, and mold ($p < 0.001$). Tukey's HSD test ($p < 0.05$) revealed significant differences between control and marinated treatments across storage times, while variations among different marination recipes were not significant.

*Total Aerobic Bacteria, Coliform, and E. coli.*

The total aerobic bacterial count reflects the overall microbial load of food products and is widely used as an indicator of hygienic quality, spoilage potential, and storage stability, although it does not directly correlate with specific food safety hazards (BPOM, 2012). In the present study, unmarinated tuna and nile tilapia exhibited high aerobic bacterial loads, ranging from 132 x $10^5$ CFU/g to 205 x $10^5$ CFU/g, with nile tilapia consistently showing higher values than tuna across all storage times (Figure 3; Table 1). These findings suggest that nile tilapia may be more susceptible to microbial spoilage compared to tuna. The decreases in the number of microbes in fish treated with marination has been caused by antibacterial activity of herbs and spices against a range of gram-positive and gram-negative bacteria, including pathogens of the gastrointestinal-tract (Mapeka et al., 2024). According to Tukey's HSD test (Table 1), nile tilapia consistently clustered in higher significance group (a-c) than tuna, highlighting species-specific differences in susceptibility to microbial spoilage during storage.

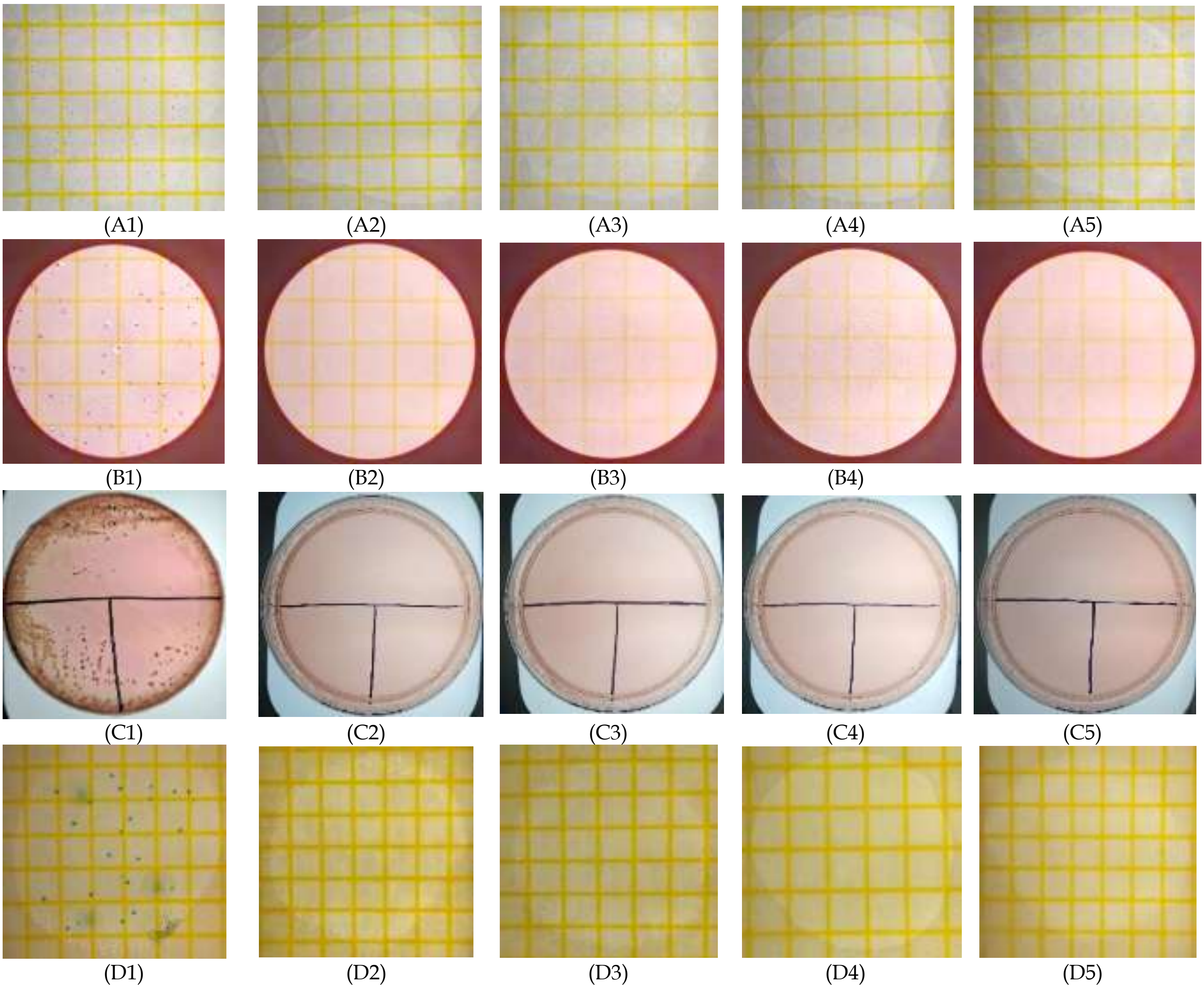


**Figure 3.** (A1) Unmarinated fish at AC plate, (A2) Marinated fish at AC plate one hours of marination, (A3) Marinated fish at AC plate three days of marination, (A4) Marinated fish at AC plate five days of marination, (A5) Marinated fish at AC plate seven days of marination, (B1) Unmarinated fish at EC plate, (B2) Marinated fish at EC plate one hours of marination, (B3) Marinated fish at EC plate three days of marination, (B4) Marinated fish at EC plate five days of marination, (B5) Marinated fish at EC plate seven days of marination, (C1) Unmarinated fish at SSA medium, (C2) Marinated fish at SSA medium one hours of marination, (C3) Marinated fish at SSA medium three days of marination, (C4) Marinated fish at SSA medium five days of marination, (C5) Marinated fish at SSA medium seven days of marination, (D1) Unmarinated fish at RYM plate, (D2) Marinated fish at RYM plate one hours of marination, (D3) Marinated fish at RYM plate three days of marination, (D4) Marinated fish at RYM plate five days of marination, (D5) Marinated fish at RYM plate seven days of marination

In contrast, all marinated fish (TU1-3, TK1-3, NU1-3, NK1-3) maintained extremely low bacterial loads, in the range of 0-2 x $10^5$ CFU/g, grouped within categories I and j (Table 1). These values were significantly lower ($p < 0.05$) than controls and showed no significant variation ($p > 0.05$) across different recipes or storage times, indicating the consistent antimicrobial efficacy of spice-based marinades. This suppression of microbial growth can be attributed to the antimicrobial activity of bioactive compounds present in spices and herbs used in the marinade. Previous studies have shown that compounds such as carvacrol in oregano disrupt bacterial membrane integrity, causing destabilization and leakage of intracellular material, while essential oils from lemongrass induce cell lysis, inhibit septum formation, and cause morphological abnormalities in

Gram-positive and Gram-negative bacteria (Faheem et al., 2022). Together, these mechanisms explain the pronounced reduction of microbial growth in marinated fish.

*Coliforms* serve as important indicators of sanitary quality due to their association with fecal and environmental contamination. Members of this group include Citrobacter, Enterobacter, Escherichia, Klebsiella, Serratia, and Hafnia (Eden, 2014). In the control samples, *Coliform* counts increased significantly ($p < 0.05$) over storage duration (Table 1). Tuna rose from 32 x $10^5$ CFU/g at D0 to 55 x $10^5$ CFU/g at D7, while nile tilapia showed higher counts, ranging from 81 x $10^5$ CFU/g to 88 x $10^5$ CFU/g. By contrast, nearly all marinated treatments showed no detectable *Coliforms* or only trace levels (0–1 x $10^5$ CFU/g). These findings highlight the effectiveness of spice-based marinades in suppressing coliform growth and maintaining sanitary quality during storage.

**Table 1.** Tukey's HSD of Number of Aerobic Bacteria, *Coliform*, *E. coli*, *Salmonella*, *Shigela*, Yeast, and Mold

| Treatment | Storage time | Number of Aerobic Bacteria* | Number of *Coliform** | Number of *E. coli** | Number of *Salmonella** | Number of *Shigella** | Number of Yeast* | Number of Mold* |
|---|---|---|---|---|---|---|---|---|
| TC | D0 | 132 h | 32 g | 7 d | 0 | 21 f | 9 b | 4 e |
| TC | D3 | 152 g | 39 f | 8 d | 0 | 29 d | 9 b | 6 d |
| TC | D5 | 160 f | 42 e | 9 c | 0 | 28 e | 10 b | 7 c |
| TC | D7 | 174 d | 55 d | 11 b | 0 | 37 c | 11 a | 10 b |
| NC | D0 | 172 e | 81 c | 9 c | 27 c | 12 h | 12 a | 5 d |
| NC | D3 | 183 c | 80 c | 7 d | 23 d | 14 g | 10 b | 7 c |
| NC | D5 | 186 b | 86 b | 10 b | 28 b | 180 b | 9 b | 9 b |
| NC | D7 | 205 a | 88 a | 12 a | 31 a | 201 a | 13 a | 13 a |
| TU1 | D0 | 1 j | 1 i | 0 e | 0 e | 1 i | 1 d | 1 f |
| TU1 | D3 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU1 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU1 | D7 | 1 j | 1 i | 0 e | 0 e | 1 i | 0 d | 0 f |
| TK1 | D0 | 0 j | 1 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK1 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK1 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK1 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU1 | D0 | 1 j | 3 h | 1 e | 0 e | 0 i | 2 c | 1 f |
| NU1 | D3 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU1 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU1 | D7 | 1 j | 1 i | 0 e | 0 e | 1 i | 1 d | 0 f |
| NK1 | D0 | 2 i | 1 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK1 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK1 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK1 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 1 f |
| TU2 | D0 | 1 j | 1 i | 0 e | 0 e | 0 i | 1 d | 0 f |
| TU2 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU2 | D5 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU2 | D7 | 0 j | 1 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK2 | D0 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK2 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK2 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK2 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU2 | D0 | 1 j | 2 h | 1 e | 0 e | 1 i | 1 d | 1 f |
| NU2 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU2 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU2 | D7 | 1 j | 1 i | 0 e | 0 e | 1 i | 0 d | 1 f |
| NK2 | D0 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK2 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK2 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK2 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU3 | D0 | 1 j | 0 i | 0 e | 0 e | 0 i | 1 d | 0 f |
| TU3 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU3 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TU3 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 1 d | 0 f |
| TK3 | D0 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |

| Treatment | Storage time | Number of Aerobic Bacteria* | Number of *Coliform** | Number of *E. coli** | Number of *Salmonella** | Number of *Shigella** | Number of Yeast* | Number of Mold* |
|---|---|---|---|---|---|---|---|---|
| TK3 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK3 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| TK3 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU3 | D0 | 2 i | 2 h | 0 e | 0 e | 1 i | 1 d | 1 f |
| NU3 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU3 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NU3 | D7 | 1 j | 1 i | 0 e | 0 e | 1 i | 1 d | 0 f |
| NK3 | D0 | 1 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK3 | D3 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK3 | D5 | 0 j | 0 i | 0 e | 0 e | 0 i | 0 d | 0 f |
| NK3 | D7 | 0 j | 0 i | 0 e | 0 e | 0 i | 1 d | 0 f |

*Number of colony (x $10^5$ CFU/g)

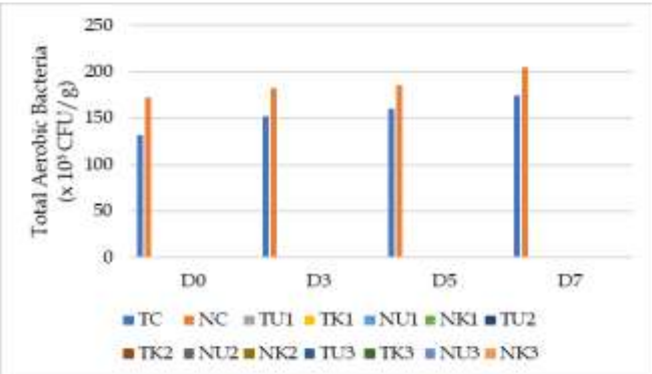


**Figure 4**. Total aerobic bacteria (x $10^5$ CFU/g) during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

*E. coli*, another important indicator of contamination and potential pathogenic risk (Mulyati et al., 2024), was present at low but detectable levels in controls. In tuna, counts increased slightly from 7 x $10^5$ CFU/g at D0 (group d) to 11 x $10^5$ CFU/g at D7 (group b), while nile tilapia showed higher levels from 9 x $10^5$ CFU/g at D0 (group c) to 12 x $10^5$ CFU/g at D7 (group a). Accordingly, nile tilapia consistently ranked in higher significance groups compared to tuna (Table 1). Marinated treatments, by contrast, showed complete absence of *E. coli* (0 to 1 x $10^5$ CFU/g) throughout the experiment, regardless of recipe or storage duration (Figure 5). Statistical comparisons confirmed a highly significant reduction ($p < 0.001$) between marinated and unmarinated fish samples. The visual separation of colony counts between unmarinated and marinated samples is also clearly illustrated in Figure 1, where unmarinated fish plates show dense microbial growth compared to the sparsely populated marinated treatments.

The antibacterial activity of spices is primarily attributed to phenolic compounds that interact with bacterial cell structures through hydrophobic and hydrogen bonding with membrane proteins, leading to membrane disruption, impairment of the electron transport system, and cell wall damage (Juglal et al., 2002). Turmeric has demonstrated antibacterial potential against selected microorganisms and may be valuable for pharmaceutical applications, particularly in drug development and in regulating abnormal serum lipid profiles. The thick structural components of Gram-positive organisms such as *Bacillus* spp. and *Staphylococcus aureus*, as well as Gram-negative *E. coli*, may facilitate stronger interactions between curcumin and bacterial lipoproteins (Odo et al., 2023).

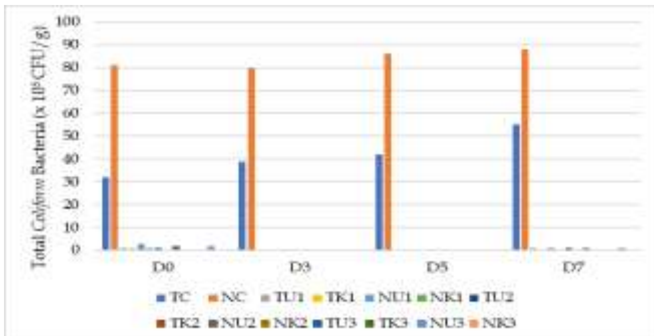


**Figure 5**. Total *Coliform* bacteria (x $10^5$ CFU/g) during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

Lemon has essential bioactive compound that showed good antibacterial efficacy and the highest zone of inhibition of *E. coli* (Sengupta et al., 2024). Lemon has significant antibacterial activity against *Staphylococcus aureus*, a gram-positive pathogen that easily contaminates food and also preventing the growth of pathogenic bacteria, particularly *Listeria monocytogenes* (Magalhães et al., 2023). *M. koenigii* (curry leaf) predominantly contains alkaloids, terpenoids, saponins, flavonoids, and tannins, all of which exhibit antibacterial activity (Syaifurrisal et al., 2024). Curry leaf also has antibacterial properties against *Salmonella* sp. *Bacillus* sp., *S. aureus*, *Candida albicans* and *E. coli* (Oluchi et al., 2025). Cumin has antibacterial effectiveness against *Porphyromonas gingivalis*, *Streptococcus mutants*, and *C. albicans* (Reni Yuslianti et al., 2025). *Asam sunti* has proven antimicrobial effects, demonstrating inhibitory activity against *S. aureus, C. albicans* and *E. coli*, whereas phytochemical compounds such as alkaloids, flavonoids, tannin, saponins, steroids, and triterpenoids are thought to contribute to this antimicrobial activity (Misrahanum et al., 2022).

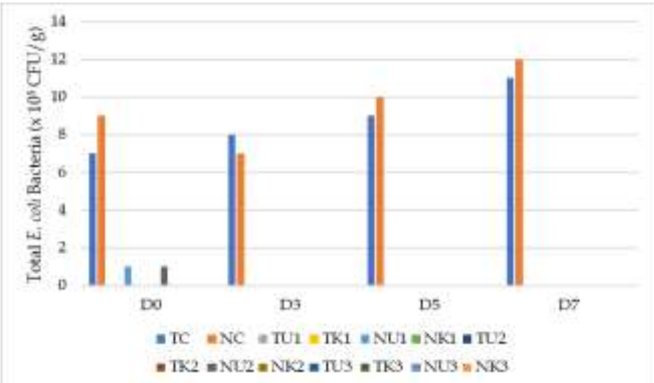


**Figure 6**. Total *E. coli* bacteria (x $10^5$ CFU/g) during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

Thyme showed antimicrobial activity against *Staphylococcus aureus* and *E. coli* (Romulo et al., 2024). Coriander showed bacterial growth inhibitory against *Staphylococcus aureus, E. coli, and Pseudomonas aeruginosa*, whereas *S. aureus* was the most sensitive bacterium to the coriander (Talebi et al., 2024). Pepper has significant amounts of bioactive compounds, particularly phenolics and flavonoids, against *E. coli* with inhibition zones ranging from 14 to 18 mm (Zhao et al., 2024).

*Total Salmonella sp. and Shigella*

Foodborne illnesses result from ingestion of toxic substances or pathogenic microorganisms in food (Mafe et al., 2024). *Salmonella* spp. represent a major concern as they can cause foodborne infection and toxico-infection (Akpoghelie et al., 2024). These bacteria have been isolated from fish viscera, gills, and skin, thereby increasing the risk of cross-contamination during handling, processing, and storage if hygienic practices and sanitation protocols are inadequate (Akinjogunla et al., 2011; Antunes et al., 2018; Dib et al., 2018).

*Salmonella* was detected exclusively in nile tilapia (27-31 x $10^5$ CFU/g) at all storage times, while no Salmonella was observed in tuna (Figure 6). This difference is likely due to ecological factors: nile tilapia are typically raised in aquaculture settings prone to contamination from livestock waste and poor water quality, while tuna were wild-cought marine fish with relatively lower exposure to *Salmonella* sources (Suleman et al., 2025). Herbal marination, with or without cooking, effectively eliminated *Salmonella* in both tuna and nile tilapia (Figure 6). Turmeric, garlic, pepper, coriander, chili, cumin, and lemongrass were able to inhibit the growth of *Salmonella* sp. bacteria which was characterized by the formation of clear zones around the disc (Jirna et al., 2020).

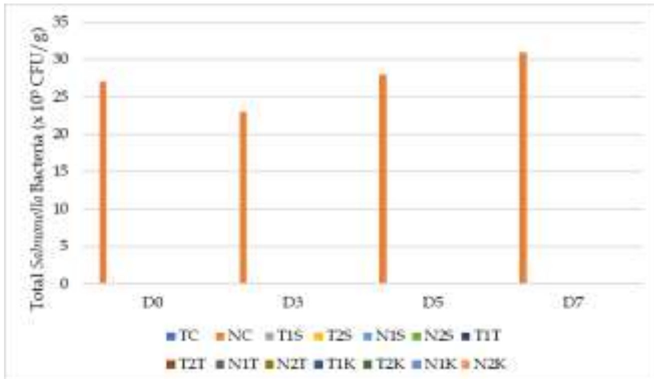


**Figure 7**. Total *Salmonella* bacteria (x $10^5$ CFU/g) during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

Lemon showed anti-*Salmonella* activity in vacuum-packed sous vide carrot samples was slightly stronger than in controls (Kačániová et al., 2024). Oregano contains compounds that cause an increase or inhibition of antimicrobial activity against bacteria that cause food poisoning such as *S. aureus* and *B. cereus*, and have very good activity against the opportunistic bacterium *S. epidermidis*, which can cause infections in immunocompromised patients, and against the absolutely pathogenic *S. typhimurium*, which causes small and large intestinal inflammation and invasive food poisoning called salmonellosis (Walasek-Janusz et al., 2024).

*Total Yeast and Mold*

Yeast and mold are major spoilage agents in fish, producing enzymes that degrade proteins and lipids, leading to off-odors, discoloration, and reduce shelf life (Darwish et al., 2023; Tahiluddin et al., 2022). Figure 8 shows that unmarinated fish contained significantly higher yeast (9-13 x $10^5$ CFU/g) (Figure 8) and mold (4-13 x $10^5$ CFU/g) (Figure 9) counts compared with marinated samples, which consistently remained below 2 x $10^5$ CFU/g for yeast (Figure 8) and 1 x $10^5$ CFU/g for mold (Figure 9).

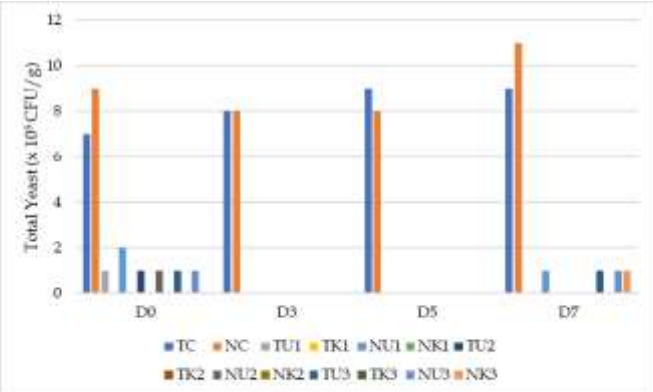


**Figure 8**. Total yeast (x $10^5$ CFU/g) during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

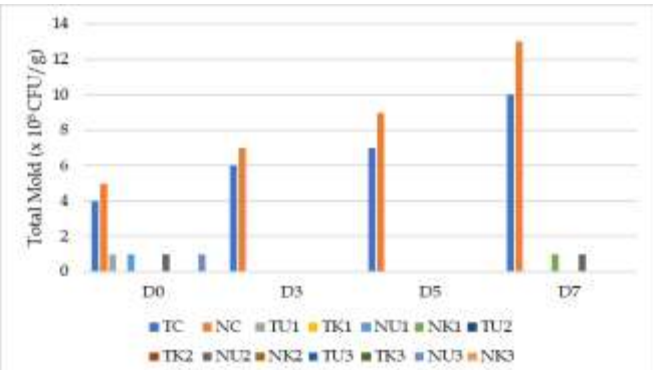


**Figure 9**. Total mold (x $10^5$ CFU/g) during 7 days of cold storage (1-4°C). Sample code: TC = tuna control; NC = nile tilapia control; TU1, TU2, TU3 = uncooked tuna recipes 1-3; TK1, TK2, TK3 = cooked tuna recipes 1-3; NU1, NU2, NU3 = uncooked nile tilapia recipe 1-3; NK1, NK2, NK3 = cooked nile tilapia recipes 1-3. Storage time: D0 = 1 hour; D3 = 3 days; D5 = 5 days; D7 = 7 days

The strong inhibition observed in marinated samples is attributable to bioactive compounds in the spices used. Phenolics and aromatics from ingredients such as thyme, oregano, and lemongrass disrupt fungal cell membranes and organelles, suppressing growth. Specifically, thyme and oregano are known to inhibit *Candida* spp. and *Penicillium digitatum* by compromising membrane integrity (Romulo et al., 2024; Walasek-Janusz et al., 2024), while lemongrass interferes with mitochondrial function and ion homeostasis, preventing conidial germination (Faheem et al., 2022). This mechanistic evidence explains the near-complete suppression of spoilage fungi in marinated fish compared with untreated controls.

The Indonesian National Standardization Agency (BSN) specifies in SNI 7388:2009 that frozen fish products must comply with microbiological safety criteria: a maximum Total Plate Count (TPC) of 5 x $10^5$ CFU/g, a maximum *E. coli* count of less than 3 MPN/g, and the absence of *Salmonella* (Furuujihim Rohsarifuddin et al., 2025). To achieve these standards, comprehensive quality control practices are necessary, including maintaining sanitation, applying preservatives, optimizing packaging, and controlling storage conditions (Mafe et al., 2024). The results of this study showed that marination with selected herbs and spices effectively reduced microbial populations, including aerobic bacteria, *Coliform, E. coli, Salmonella, Shigella*, yeast and mold, such that both tuna and nile tilapia remained within SNI microbiological limits up to seven days of storage.

## Conclusion

The results of this study indicate that marinating fish with certain spice mixtures, whether cooked or uncooked, significantly reduces microbiological contamination, including aerobic bacteria, *Escherichia coli*, *Coliforms, Salmonella, Shigella*, as well as yeast and fungi, compared to unmarinated control samples. Spices such as garlic, turmeric, lemongrass, oregano, rosemary, and lemon have strong antioxidant and antimicrobial properties, which can stop microbial growth, reduce oxidation, and maintain fish quality during storage in the refrigerator. In addition, marinating also lowers the pH and oxidation-reduction potential (ORP) of fish, creating an environment that is less conducive to microbial growth and spoilage. These findings indicate that the use of spices as natural ingredients for fish preservation is highly effective, but further research is needed to understand the long-term effects of various marinating methods and to investigate the sensory properties of marinated fish, such as taste and texture, which will enrich our understanding of the effectiveness of this preservation technique in the food industry.

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**Author Contributions**

Conceptualization, H.W. and A.L.; methodology, H.W.; software, H.W.; validation, H.W. and A.L.; formal analysis, H.W.; investigation, H.W.; resources, H.W.; data curation, H.W.; writing—original draft preparation, H.W.; writing—review and editing, H.W. and A.L; visualization, H.W.;

supervision, H.W. and A.L; project administration, H.W.; funding acquisition, H.W. and A.L. All authors have read and agreed to the published version of the manuscript.

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**Conflicts of Interest**
The authors declare no conflict of interest.

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