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Effect of Moringa Leaf and Seaweed Extract Immersion on the Organoleptic and Microbial Quality of Anchovy (*Stolephorus spp.*)

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Abstract: The small size of anchovy (*Stolephorus spp*) makes the fish easily torn and highly susceptible to quality deterioration, particularly when stored in fresh conditions. Moringa leaves (terrestrial product) and seaweed (marine product) were selected as immersion materials due to their natural bioactive compounds, such as antioxidants and antimicrobials, and to compare the most effective extract in maintaining fish freshness. The study aimed to analyze the effect of immersion duration and the effectiveness of the extracts. Immersion was conducted at room temperature for 4, 6, and 8 hours to determine the most effective duration in preserving fish quality. A concentration of 100% was applied to maximize the utilization of bioactive compounds. The experimental methodology compared anchovy samples treated with moringa leaf and seaweed extracts against untreated control samples, using a randomized block design (RBD) to minimize bias, with three replications to ensure reliability and statistical validity. Organoleptic evaluation indicated that the most effective immersion extracts were seaweed and moringa leaf extracts, with sensory scores of 8, characterized by clear eyes, red gills, transparent mucus, reddish flesh, fresh odor, and firm-elastic texture. The total microbial counts were 5.2 log CFU/g for seaweed extract and 5.32 log CFU/g for moringa leaf extract.

Introduction

Anchovy *(Stolephorus commersonnii)* is a pelagic fish belonging to the family Engraulidae. This species, also known as Commerson's anchovy (1), has a small, elongated body with a slightly bulging abdomen. It is easily recognized by its size and the distinctive silvery-white lateral line extending from head to tail. Anchovies are commonly found in coastal areas and estuaries at depths ranging from 0 to 50 m below sea level (2).

Anchovies are rich in nutrients, including carbohydrates, fats, proteins, calcium, minerals, and vitamins, but also contain a high water content of approximately 70–80%. This high moisture level makes the fish highly perishable and prone to rapid deterioration or decomposition after harvest. The elevated water content provides a favorable environment for microbial growth, which accelerates physical and chemical changes, rendering the fish unsuitable for consumption. Therefore, proper, rapid, and effective handling is essential to maintain quality. Microorganisms present in fish products can quickly induce spoilage, leading to significant declines in safety and edibility (3).

Post-harvest deterioration in fishery products, including anchovy, is mainly caused by microbial activity and biochemical changes that result in rapid quality loss. Several studies have reported that chemical preservatives such as formalin are still used to delay spoilage, despite posing serious health risks to consumers. Consequently, increasing attention has been directed toward the development of natural preservatives as safer alternatives for fish preservation. Previous studies have demonstrated that natural extracts derived from plant and marine sources exhibit antibacterial activity and have potential application in maintaining fish freshness and safety (4 - 7).

Anchovy is commonly preserved through drying. Sun-assisted drying, however, may induce photo-oxidation in fishery products such as anchovy. This process affects the water activity (Aw) and moisture content of the product. Lipid oxidation is influenced by the reduction of water content and Aw during storage, leading to changes in texture, flavor, color, and nutritional composition. As lipid oxidation progresses, anchovy quality deteriorates, making the product less suitable for consumption. Therefore, appropriate measures are required to maintain the freshness and safety of anchovy for human consumption (8).

Moringa leaves (*Moringa oleifera*) and seaweed (*Gracilaria spp.*) were selected as natural materials in this study due to their known bioactive compounds with antimicrobial properties. Moringa leaves are rich in flavonoids, phenols, and antioxidants that inhibit microbial growth through bacteriostatic mechanisms (9). Meanwhile, Gracilaria seaweed contains polysaccharides, pigments, and phenolic compounds that also exhibit antimicrobial activity and can slow down oxidation processes (10). By utilizing extracts from terrestrial (moringa leaves) and marine (seaweed Gracilaria) sources, this study aims to compare the effectiveness of both bioactive materials in preserving the quality of fresh anchovy, thereby providing an environmentally friendly and sustainable alternative for natural preservation.

This study aims to evaluate the effect of immersion treatments and to compare the effectiveness of moringa leaf extract, seaweed extract, and their combination in maintaining the freshness quality of anchovy (*Stolephorus spp.*). The objective is to determine the most effective extract and immersion duration in preserving physical freshness attributes and reducing microbial counts, thereby providing a natural preservation approach for anchovy.

Methodology

Study Design

This study employed a completely randomized experimental design to evaluate the effect of immersion duration in natural plant-based extracts on the freshness quality of anchovy fish (*Stolephorus commersonnii*). The primary objective was to assess the antimicrobial and organoleptic preservation potential of *Moringa oleifera* (Moringa) leaf extract, *Gracilaria verrucosa* (red seaweed) extract, and a combination of both, applied over varying immersion times. This approach was chosen to simulate practical, low-cost preservation methods applicable to artisanal fisheries in tropical environments.

Sample Collection and Preparation

Fresh anchovy fish (*Stolephorus commersonnii*) were collected from the East Ring Coastal Fishing Port (TPI Lingkar Timur) in Sidoarjo, East Java, Indonesia. Only specimens exhibiting uniform size (approximately 6–8 cm in length), intact morphology, and no prior exposure to preservation treatments were selected to ensure consistency across experimental units. The fish were immediately placed in insulated containers maintained at approximately 4°C to minimize microbial activity during transport. All samples were processed within two h of collection to preserve their native freshness and microbiological integrity prior to experimental treatment.

The moringa leaves *(Moringa oleifera*) used in this study were collected from trees growing around Tanggulangin Village, Sidoarjo Regency. Morphologically, the leaves are oval to elliptical, measuring 1–3 cm in length and approximately 1 cm in width, with a fresh green color and compound arrangement on the petiole. The selected samples consisted of healthy young to mature leaves, free from damage, pests, or disease spots. The leaves are thin yet firm, not wilted, and possess a characteristic herbal aroma. These physical characteristics reflect the presence of key bioactive compounds, including flavonoids, phenols, vitamins, and antioxidants, which are known to contribute to antimicrobial activity and preservation of food quality.

The *Gracilaria* seaweed used in this study was obtained directly from farmers in Jabon Village, Sidoarjo, East Java. Samples were collected fresh, immediately after harvest, ensuring the retention of bioactive compounds. The thallus exhibited a predominantly dark coloration with subtle reddish-purple hues, indicating the presence of natural pigments such as phycoerythrin and phycocyanin, which are characteristic of the *Gracilaria* genus. The thallus texture was firm and elastic, reflecting post-harvest freshness and the preservation of polysaccharide content.

The experimental design comprised twelve treatment groups formed by the combination of three extract types, *Moringa oleifera* leaf extract, *Gracilaria verrucosa* seaweed extract, and a 1:1 volumetric blend of both, with four immersion durations (0 h as control, 4 h, 6 h, and 8 h). Each treatment group was prepared in triplicate, resulting in a total of 36 independent experimental units.

Preparation of Plant Extracts

The preparation of the natural preservatives involved separate extraction processes for *Moringa oleifera* leaves and *Gracilaria verrucosa* seaweed. Fresh Moringa leaves were first thoroughly rinsed with distilled water to eliminate surface contaminants, then shade-dried for 48 h at ambient temperature (27 ± 2°C) until a consistent dryness was achieved. The dried leaves were subsequently ground into a fine powder. To produce the aqueous extract, 100 grams of the powdered leaves were blended with 1 liter of sterile distilled water and the mixture was filtered sequentially through muslin cloth and Whatman No.1 filter paper. The resulting filtrate was stored at 4°C and used within 24 h to preserve its bioactive properties.

Similarly, *Gracilaria verrucosa* seaweed samples were washed to remove impurities, sun-dried, and pulverized into powder form. The extraction process followed the same protocol as for Moringa, with 100 grams of seaweed powder dissolved in 1 liter of sterile distilled water and filtered in the same manner. For the combination treatment, equal volumes of the Moringa and seaweed extracts were mixed immediately prior to application to ensure homogeneity and maximize potential synergistic effects.

Immersion Procedure

Anchovy samples (150 g per replicate) were immersed in 100% (undiluted) extract solutions for 4, 6, or 8 h at ambient laboratory temperature (28 ± 1°C). Control samples were not immersed. All treatments were conducted under aseptic conditions, and after immersion, samples were drained for 15 min on sterile stainless-steel mesh trays before further testing.

Organoleptic Assessment

Organoleptic evaluation followed the Indonesian National Standard (SNI 01-2346-2006) for fresh fish, using six criteria: eye clarity, gill color, mucus presence, flesh texture, odor, and firmness. A panel of 30 untrained individuals rated each sample using a 9-point hedonic scale (1 = extremely poor, 9 = extremely fresh). Panelists were blind to treatment conditions, and all assessments were conducted under consistent lighting and temperature.

Microbiological Analysis

Total microbial load was quantified using the Total Plate Count (TPC) method in accordance with SNI 2332.3:2015. Fish samples (25 g) were aseptically homogenized with 225 mL of sterile buffered peptone water, serially diluted, and plated on Plate Count Agar. Plates were incubated at 35°C for 48 h, and colony-forming units (CFU) were counted. Results were expressed as log CFU/g.

Data Analysis

Statistical analysis was conducted using SPSS version 26.0. Two-way analysis of variance (ANOVA) was used to evaluate the effects of extract type and immersion duration on organoleptic scores and microbial counts. When significant differences were detected ($p < 0.05$), Duncan's multiple range test was applied for post-hoc comparisons. Data normality and homoscedasticity were verified using Shapiro-Wilk and Levene's tests, respectively.

Ethical Considerations

As this study did not involve human or vertebrate animal experimentation, formal ethical approval was not required. However, all procedures involving microbial handling and food sample testing adhered strictly to institutional biosafety guidelines and food hygiene regulations.

Results

Organoleptic Evaluation of Anchovy Fish (*Stolephorus commersonnii*)

Organoleptic analysis was conducted to assess the sensory freshness of anchovy samples subjected to different soaking treatments (11, 12). These included immersion in *Moringa oleifera* leaf extract (P1), *Gracilaria verrucosa* seaweed extract (P2), a combination of both extracts (P3), formalin (P+; positive control), and untreated fish (P0; negative control). Six quality parameters were evaluated: eye clarity, gill color, mucus condition, flesh texture, odor, and firmness. The average scores across treatments and immersion durations (0, 4, 6, and 8 h) are presented in **Table 1**.

All fresh samples initially met the minimum sensory quality threshold of 7.0, as defined by the Indonesian National Standard (SNI 01-2346-2006) (13). However, a gradual decline in organoleptic scores was observed with increased immersion time, particularly in the untreated group (P0). By contrast, samples treated with seaweed extract (P2) and the combined extract (P3) retained higher scores at 6 and 8 h, particularly in odor and texture parameters.

One-way ANOVA revealed a statistically significant difference in mean organoleptic scores among treatments ($F = 6.571$; $p = 0.014$), as shown in **Table 2**. Duncan's multiple range test further identified group differences, indicating that treatments P2, P3, and P+ maintained significantly higher sensory quality than the untreated control (P0) (see **Table 3**).

Eye Clarity Parameter

Eye clarity is one of the most immediate indicators of fish freshness perceived by consumers (14). Organoleptic assessment of eye clarity in anchovies subjected to different soaking treatments over various durations is summarized in **Table 4**.

Among all treatments, P2 (seaweed extract) consistently maintained the highest eye clarity scores, particularly at 6 and 8 h, indicating superior freshness retention. Remarkably, its performance surpassed that of formalin, suggesting that seaweed extract may serve as a more natural and effective preservative for anchovy.

Statistical analysis using one-way ANOVA (see **Table 5**) confirmed a significant effect of treatment on eye clarity scores ($p = 0.014$). Post-hoc Duncan's test revealed that P2, P3, and P+ had significantly higher mean values than P0.

Table 1. Mean organoleptic scores of anchovy (Stolephorus commersonnii) across treatments and immersion durations.

Time (Hours)	Treatment	Eyes	Gills	Mucus	Flesh	Odor	Texture
0	P0	8.4	8.5	8.0	8.3	8.3	8.2
	P1	8.4	8.4	8.4	8.3	8.2	8.2
	P2	8.3	8.4	8.1	8.4	8.3	8.3
	P3	8.4	8.3	8.2	8.3	8.3	8.3
	P+	7.4	7.5	7.0	7.6	7.8	7.7
4	P0	8.1	8.1	8.0	8.1	7.9	8.1
	P1	8.2	8.1	7.8	7.7	7.6	7.6
	P2	8.2	8.1	8.2	8.2	8.1	8.0
	P3	8.2	8.1	8.2	8.2	8.1	8.2
	P+	8.2	8.2	8.1	8.4	8.1	8.3
6	P0	7.2	7.0	7.0	7.1	6.9	7.1
	P1	7.6	7.3	7.1	7.1	7.1	7.2
	P2	7.8	7.7	7.6	7.6	7.4	7.5
	P3	7.8	8.1	8.0	7.9	7.7	8.0
	P+	8.2	8.1	7.8	8.4	7.4	8.1
8	P0	6.0	6.0	6.0	5.3	5.6	5.0
	P1	6.9	7.0	6.0	6.7	6.8	6.6
	P2	7.8	7.5	7.5	7.5	7.3	7.4
	P3	7.3	7.7	7.8	7.7	7.4	7.6
	P+	7.5	7.6	7.5	8.3	7.3	8.0

Note: P0 = Untreated (control), P1 = *Moringa oleifera* extract, P2 = *Gracilaria verrucosa* extract, P3 = Combined Moringa and seaweed extract, P+ = formalin (positive control). Minimum organoleptic score for fresh fish = 7.0 (SNI 01-2346-2006).

Table 2. One-way ANOVA results for organoleptic scores of anchovy.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.448	4	1.122	6.571	0.014
Within Groups	12.456	120	0.311		
Total	16.903	124			

Table 3. Duncan's multiple range test results for anchovy organoleptic scores.

Treatment	N	Subset for α = 0.05	
		1	2
P0	40	7.1111	
P1	40	7.5474	7.5474
P2	40		7.9400
P3	40		7.7779
P+	40		7.9889
Sig.		0.107	0.130

Table 4. Mean eye clarity scores of anchovy across treatments and immersion durations.

Treatment	0 h	4 h	6 h	8 h
P0	7.4	8.1	7.2	6.0
P1	8.4	8.2	7.6	6.9
P2	8.3	8.2	7.8	7.8
P3	8.4	8.2	7.8	7.3
P+	8.4	8.2	8.2	7.5

Note: P0 = Untreated control; P1 = *Moringa oleifera* extract; P2 = *Gracilaria verrucosa* extract; P3 = Combination of Moringa and seaweed extract; P+ = Formalin (positive control).

Table 5. One-way ANOVA results for anchovy eye organoleptic scores.

Source	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	4.448	4	1.112	3.571	0.014
Within Groups	12.456	40	0.311		
Total	16.903	44			

Table 6. Mean gill scores of anchovy fish across treatments and immersion durations.

Treatment	0 h	4 h	6 h	8 h
P0	7.5	8.1	7.0	6.0
P1	8.4	8.1	7.3	7.0
P2	8.4	8.1	7.7	7.5
P3	8.3	8.1	8.1	7.7
P+	8.4	8.2	8.1	7.6

Note: P0 = Untreated (control); P1 = *Moringa oleifera* extract; P2 = *Gracilaria verrucosa* extract; P3 = Combination of Moringa and seaweed extract; P+ = Formalin (positive control).

Gill Parameter

Gills are a primary site for microbial activity in fish due to their rich vascularization, making them particularly susceptible to spoilage (15). Organoleptic evaluation of anchovy gills across various soaking treatments and durations is presented in **Table 6**.

Among all treatments, P3 (combined Moringa and seaweed extract) maintained the highest average score at 8 h (7.7), slightly surpassing the formalin control (P+), which scored 7.6. These results suggest that the natural preservative combination was more effective in preserving gill freshness and was more favorably evaluated by panelists than the synthetic formalin treatment, particularly in maintaining color, firmness, and overall visual acceptability during extended soaking periods. The one-way ANOVA analysis (see **Table 7**) confirmed a significant difference between treatments ($p = 0.001$), indicating that the type of soaking treatment had a statistically significant impact on gill quality. Duncan's multiple range test (see **Tables 8 and 9**) further differentiated the treatments into distinct subsets, with P3 and P+ forming the top-performing group.

Mucus Parameter

Fish mucus provides a favorable environment for bacterial proliferation due to its high moisture and nutrient content (16). The organoleptic assessment of anchovy mucus across various soaking treatments and durations at room temperature is summarized in **Table 10**.

At 8 h of immersion, the highest mucus score was observed in P3 (7.8), slightly higher than P+ (7.5). This indicates that the combination of Moringa and seaweed extracts was more effective in maintaining mucus quality than the synthetic formalin treatment and was more favorably evaluated by panelists.

One-way ANOVA (see **Table 11**) showed a significant difference among treatments ($p < 0.001$), confirming that soaking treatment had a significant effect on mucus quality. Duncan's test (see **Table 12**) futher grouped the treatments, with P3 and P+ yielding the highest mean scores.

Flesh Parameter

The flesh of fish is a primary indicator of freshness, as it directly reflects physical integrity and spoilage progression (17). Organoleptic evaluation of anchovy flesh under various soaking treatments at room temperature, see **Table 13**.

At 8 h of immersion, the highest flesh score was recorded in the formalin-treated group (P+), with a consistent score of 8.3. This value exceeded that of the natural preservative treatment (P3), which scored 7.7. These findings suggest that while natural extracts maintained flesh quality well, formalin remained the most effective in preserving texture.

The use of seaweed and moringa leaf extracts as natural preservatives has been proven to maintain the physical quality of anchovy (*Stolephorus spp.*). Sulfated polysaccharides from seaweed form a protective layer that preserves texture, while marine pigments act as antioxidants that prevent lipid oxidation. Flavonoids and phenolic compounds from moringa leaves exhibit bacteriostatic properties, suppressing the growth of spoilage microorganisms while maintaining the color and aroma of the fish. The combination of both extracts produces a synergistic effect, in which the bactericidal activity of seaweed and the bacteriostatic activity of moringa leaves work together to extend the freshness of anchovy without the use of synthetic preservatives.

Table 7. One-way ANOVA results for anchovy gill organoleptic scores.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.996	4	1.499	5.757	0.001
Within Groups	10.416	40	0.260		
Total	16.412	44			

Note: Differences among treatments are significant at $p < 0.05$.

Table 8. Duncan's multiple range test results for anchovy gill scores.

Treatment	N	Subset for α = 0.05		
		1	2	3
P0	9	7.0333		
P1	9	7.4444	7.4444	
P2	9		7.8556	7.8556
P3	9			7.9889
P+	9		7.9444	7.9444
Sig.		0.095	0.055	0.606

Table 9. Duncan's multiple range test results for anchovy eye scores.

Treatment	N	Subset for α = 0.05	
		1	2
P0	9	7.1111	
P1	9	7.5444	7.5444
P2	9		7.9000
P3	9		7.7778
P+	9		7.9889
Sig.		0.107	0.130

Table 10. Mean mucus scores of anchovy fish across treatments and immersion durations.

Treatment	0 h	4 h	6 h	8 h
P0	7.0	8.0	7.0	6.0
P1	8.4	7.8	7.1	6.0
P2	8.1	8.2	7.6	7.5
P3	8.2	8.2	8.0	7.8
P+	8.2	8.1	7.8	7.5

Note: P0 = Untreated (control); P1 = *Moringa oleifera* extract; P2 = *Gracilaria verrucosa* extract; P3 = Combination of Moringa and seaweed extract; P+ = Formalin (positive control).

Table 11. One-way ANOVA results for anchovy mucus organoleptic scores.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	6.555	4	1.639	7.208	0.000
Within Groups	9.093	40	0.227		
Total	15.648	44			

The one-way ANOVA (see **Table 14**) indicated a statistically significant difference among treatments ($p < 0.001$), affirming that soaking treatments had a significant effect on flesh quality. Duncan's test (see **Table 15**) further revealed clear groupings, with P+ and P3 forming the highest-performing clusters.

Odor Parameter

Odor is one of the most immediate and perceptible indicators of fish freshness, making it a critical parameter in organoleptic assessment (18). The average odor scores of anchovy samples subjected to different soaking treatments at room temperature are presented in **Table 16**.

At the 8-hour mark, the highest odor score was observed in the P3 treatment (7.4), slightly exceeding the formalin treatment (P+, 7.3). This suggests that natural preservatives, specifically the combination of Moringa and seaweed extracts, may be more effective in maintaining odor freshness than formalin.

The combination of seaweed and moringa leaf extracts is effective in maintaining the aroma quality of anchovy (*Stolephorus spp.*) during storage. Sulfated polysaccharides present in seaweed are able to form a protective layer on the fish surface, which inhibits lipid oxidation processes and consequently prevents the development of rancid odors associated with spoilage. Meanwhile, flavonoids and phenolic compounds derived from moringa leaves function as natural antioxidants and also contribute a mild herbal aroma that helps neutralize the characteristic fishy odor of seaweed-treated anchovy. The application of these natural extracts therefore produces anchovy products with a fresher, cleaner, and more stable aroma profile throughout storage, without reliance on synthetic preservatives. As a result, the overall sensory quality is enhanced and short-term shelf acceptability under room temperature conditions is effectively extended.

One-way ANOVA analysis (see **Table 17**) revealed a statistically significant difference among treatments ($p = 0.005$), confirming that odor scores were significantly influenced by the type of immersion. Duncan's post-hoc test (see **Table 18**) supported these findings, grouping P3 and P+ in the highest scoring subsets.

Texture Parameter

Texture is a critical determinant of consumer preference, reflecting the structural integrity and freshness of fish (19). It enables sensory perception of firmness, elasticity, and physical resistance during consumption, which are closely associated with post-harvest quality deterioration and handling conditions, particularly those related to moisture loss, protein denaturation, and microbial activity during storage. The organoleptic scores for anchovy texture across various immersion treatments at room temperature are summarized in **Table 19**.

After 8 h, the highest texture score was observed in the formalin treatment group (P+), indicating superior elasticity and firmness compared to the natural extract treatments. Among natural alternatives, the combination of Moringa and seaweed (P3) also showed relatively high texture retention.

The one-way ANOVA (see **Table 20**) revealed a statistically significant difference among treatments ($p = 0.003$), confirming that immersion solutions significantly influenced the textural quality. Duncan's post-hoc analysis (see **Table 21**) further identified P+ and P3 as the top-performing treatment groups.

Table 12. Duncan's multiple range test results for anchovy mucus scores.

Treatment	N	Subset for α = 0.05	
		1	2
P0	9	6.9889	
P1	9	7.2333	
P2	9		7.7667
P3	9		7.9889
P+	9		7.8222
Sig.		0.283	0.358

Table 13. Mean flesh scores of anchovy fish across treatments and immersion durations.

Treatment	0 h	4 h	6 h	8 h
P0	7.6	8.1	7.1	5.3
P1	8.3	7.7	7.1	6.7
P2	8.4	8.2	7.6	7.5
P3	8.3	8.2	7.9	7.7
P+	8.3	8.4	8.4	8.3

Note: P0 = Untreated (control); P1 = *Moringa oleifera* extract; P2 = *Gracilaria verrucosa* extract; P3 = Combination of Moringa and seaweed extract; P+ = Formalin (positive control).

Table 14. One-way ANOVA results for anchovy flesh organoleptic scores.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	12.836	4	3.209	8.503	0.000
Within Groups	15.096	40	0.377		
Total	27.932	44			

Table 15. Duncan's multiple range test results for anchovy flesh scores.

Treatment	N	Subset for α = 0.05		
		1	2	3
P0	9	6.8444		
P1	9	7.2111	7.2111	
P2	9		7.7556	7.7556
P3	9			7.9333
P+	9			8.3556
Sig.		0.213	0.067	0.056

Table 16. Mean odor scores of anchovy fish across treatments and immersion durations.

Treatment	0 h	4 h	6 h	8 h
P0	7.8	7.9	6.9	5.6
P1	8.2	7.6	7.1	6.8
P2	8.3	8.1	7.4	7.3
P3	8.3	8.1	7.7	7.4
P+	8.2	8.1	7.4	7.3

Total Microbial Count of Anchovy (*Stolephorus commersonnii*) Treated with *Moringa oleifera* Leaf Extract

Total Plate Count (TPC) analysis was conducted to evaluate the antimicrobial efficacy of *Moringa oleifera* leaf extract compared to formalin and untreated controls under room temperature conditions (20). The results are summarized in **Table 22**.

Despite showing a reduction in microbial load compared to the untreated control (P0), the Moringa extract treatment (P1) was less effective than formalin (P+), which completely inhibited microbial growth by hour 8 under the experimental conditions applied. These results suggest that while *Moringa oleifera* extract exhibits antimicrobial properties, it may not achieve the same sterilizing effect or rapid microbial suppression as synthetic preservatives like formalin.

Moringa leaf extract exhibits antimicrobial activity through mechanisms that inhibit bacterial metabolism and growth, primarily by interfering with enzymatic processes and essential cellular functions involved in microbial survival. The bacteriostatic properties of moringa leaves are effective in suppressing microbial populations and slowing the progression of spoilage during storage; however, these effects are generally insufficient to completely eliminate all microorganisms under typical storage conditions. In contrast, synthetic preservatives such as formalin act by destructing bacterial proteins and nucleic acids, thereby disrupting vital structural and genetic components, which results in a strong bactericidal effect capable of significantly reducing or eradicating the entire microbial population.

The one-way ANOVA revealed a statistically significant difference among treatments (p = 0.001). Duncan's post-hoc test (see **Table 22**) confirmed that formalin-treated samples had significantly lower microbial counts than the natural extract and control groups. In **Table 22**, the results of the Duncan test are presented with superscripts *a* and *b*. In subset or superscript *a*, the treatments without immersion and with immersion in moringa leaves show no significant differences; in other words, the mean total microbial counts are the same. In subset or superscript *b*, the treatments with immersion in seaweed extract, seaweed–moringa leaf extract, and formalin also show no significant differences, indicating that the mean total microbial counts are the same.

Discussion

Organoleptic Evaluation

Overall, immersion in Moringa oleifera extract, Gracilaria verrucosa extract, and their combination exhibited a positive influence on all sensory freshness parameters, including eyes, gills, mucus, flesh, odor, and texture. The bioactive constituents of Moringa such as phenolics, flavonoids, and saponins inhibit microbial activity and slow autolysis, thereby delaying quality deterioration. Meanwhile, Gracilaria extract contains phenolic compounds, terpenoids, alkaloids, and chloramphenicol-like metabolites that exert antibacterial effects, contributing to preservation of tissue integrity and sensory attributes. When combined, these extracts complement each other, resulting in synergistic preservation where Moringa stabilizes surface biological integrity while Gracilaria enhances antimicrobial protection, producing high sensory scores across parameters. The results of this study demonstrate that natural extracts play a significant role in delaying quality deterioration in anchovy during room temperature storage.

Table 17. One-way ANOVA results for anchovy odor organoleptic scores.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	5.780	4	1.445	4.384	0.005
Within Groups	13.184	40	0.330		
Total	18.964	44			

Table 18. Duncan's multiple range test results for anchovy odor scores.

Treatment	N	Subset for α = 0.05	
		1	2
P0	9	6.7889	
P1	9	7.1667	
P2	9		7.6667
P3	9		7.7222
P+	9		7.6000
Sig.		0.170	0.066

Table 19. Mean texture scores of anchovy fish across treatments and immersion durations.

Treatment	0 h	4 h	6 h	8 h
P0	7.7	8.1	7.1	5.0
P1	8.2	7.6	7.2	6.6
P2	8.3	8.0	7.5	7.4
P3	8.3	8.2	8.0	7.6
P+	8.3	8.3	8.1	8.0

Note: P0 = Untreated (control); P1 = *Moringa oleifera* extract; P2 = *Gracilaria verrucosa* extract; P3 = Combination of Moringa and seaweed extract; P+ = Formalin (positive control).

Table 20. One-way ANOVA results for anchovy texture organoleptic scores.

Source of Variation	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	9.701	4	2.425	4.798	0.003
Within Groups	20.218	40	0.505		
Total	29.919	44			

Table 21. Duncan's multiple range test results for anchovy texture scores.

Treatment	N	Subset for α = 0.05		
		1	2	3
P0	9	6.7333		
P1	9	7.1667	7.1667	
P2	9		7.7000	7.7000
P3	9			7.9444
P+	9		7.8778	7.8778
Sig.		0.203	0.050	0.498

Table 22. Total plate count (log CFU/g) of anchovy treated.

Treatment	Observation (log Cfu/g)		
	4	6	8
P0	6,42^{a}	6,43^{a}	6,46^{a}
P1	5,36^{a}	5,40^{a}	5,45^{a}
P2	5,38^{b}	5,34^{b}	5,28^{b}
P3	5,40^{b}	5,34^{b}	5,32^{b}
P+	4,20^{b}	4,08^{b}	0

Eye Parameter

Fish eyes are reliable indicators of freshness (21). Anchovies immersed in *Moringa oleifera* extract scored 8.2 at 4 h (bright, flat eye, clear cornea), 7.6 at 6 h (slightly cloudy cornea, greyish pupil), and 6.9 at 8 h (near neutral). Decline in quality was attributed to bacterial invasion facilitated by loss of immune defense postmortem, with Moringa phenolic compounds potentially altering eye membrane permeability (22). *Gracilaria verrucosa* extract maintained higher scores: 8.2 at 4 h, 7.9 at 6 h, and 7.7 at 8 h, likely due to secondary metabolites inhibiting both Gram-positive and Gram-negative bacteria. The combination of both extracts yielded 7.3 at 8 h. Formalin-treated samples scored 7.5 at 8 h, remaining fresh due to protein cross-linking by reactive aldehyde groups. Controls declined progressively due to microbial growth. ANOVA confirmed significant treatment effects (p = 0.014).

Gill Parameter

Gills, rich in blood, are highly susceptible to bacterial spoilage (23). Moringa extract immersion yielded scores of 8.1 at 4 h, 7.3 at 6 h, and 7.0 at 8 h, all without mucus. *Gracilaria* extract scored 8.1, 7.7, and 7.5, attributed to antibacterial metabolites disrupting bacterial metabolism. The combination treatment maintained an average score of 8.0. Immersion with the combined extracts of seaweed and moringa leaves produced better results compared to single extracts. Sulfated polysaccharides from seaweed naturally formed a protective layer on the gill texture of the fish. Meanwhile, flavonoids and phenolic compounds from moringa leaves, with their bacteriostatic and antioxidant properties, effectively suppressed oxidation. As a result, the gills retained a bright coloration. Formalin-treated samples scored 8.0, though color tended towards dark red. Controls dropped to 6.0 by 8 h. ANOVA showed significant differences (p = 0.001).

Mucus Parameter

Mucus integrity reflects freshness and spoilage onset (24). Moringa extract maintained clear, transparent mucus up to 4 h, turning slightly cloudy by 6–8 h. *Gracilaria* extract preserved clear mucus throughout 8 h (scores: 8.2, 7.6, 7.5). The combination treatment scored 8.0 consistently. Formalin maintained mucus clarity (score 8.0 at 8 h). Immersion with the combined extracts of seaweed and moringa leaves yielded better results compared to single extracts. Sulfated polysaccharides from seaweed naturally formed a protective layer on the fish mucus. Meanwhile, flavonoids and phenolic compounds from moringa leaves, with their bacteriostatic and antioxidant properties, effectively suppressed oxidation. As a result, the fish mucus remained clear and free from strong odors. Controls dropped to 6.0 due to spoilage bacteria proliferating in glycoprotein-rich mucus. ANOVA indicated significant effects ($p < 0.001$).

Flesh Parameter

Moringa extract preserved flesh quality with a score of 7.0 at 8 h (slightly dull cut, intact peritoneal wall). *Gracilaria* extract achieved 7.5, and the combination 7.7, likely due to flavonoid antibacterial action inhibiting cell membrane synthesis. Formalin samples scored 8.0, with firm, elastic flesh strongly bound to the backbone. Controls declined to 5.3, showing softness and detachment due to autolysis. ANOVA confirmed significance ($p < 0.001$).

Odor Parameter

Moringa extract-treated fish scored 7.0 at 8 h (neutral odor with distinct Moringa scent from volatile antimicrobials). *Gracilaria* extract scored 7.3, with carrageenan likely masking fish odor. The combination treatment scored 7.4, balancing Moringa volatiles with odor-masking carrageenan. Formalin samples scored 7.0 with no unpleasant odor. Controls dropped to 5.6, developing ammonia and sour notes from microbial proteolysis. ANOVA showed significance ($p = 0.005$).

Texture Parameter

Moringa extract maintained acceptabletexture (score 7.0 at 8 h, slightly firm and elastic). *Gracilaria* extract scored 7.4, possibly due to chloramphenicol-like compounds inhibiting bacterial protein synthesis. The combination treatment and formalin both scored 8.0, retaining firmness and elasticity. Controls dropped to 5.0, becoming soft and less elastic due to autolysis. ANOVA confirmed significant effects ($p = 0.005$).

Total Microbial Count

Moringa Leaf Extract Immersion

Immersion of anchovies in 100% *Moringa oleifera* extract at room temperature maintained total microbial counts within the Indonesian National Standard (SNI) for fresh fish quality (25). Counts increased from 2.3×10^4 CFU/g at 4 h to 2.8×10^4 CFU/g at 8 h, indicating gradual quality decline due to the extract's bacteriostatic rather than bactericidal properties. Active compounds such as saponins, tannins, and flavonoids inhibit bacterial growth rates but do not kill bacteria (26). Decreased antimicrobial efficacy over time may be due to oxidative degradation of polyphenols, reducing antibacterial potency. Formalin immersion (1%) resulted in counts of 1.6×10^4 at 4 h, 1.2×10^4 at 6 h, and undetectablebacteria at 8 h, reflecting its strong bactericidal effect via protein cross-linking. However, formalin is unsafe for food preservation (27). ANOVA showed significant effects ($p = 0.001$), with P1 (Moringa) and P0 (control) not significantly different.

Seaweed Extract Immersion

Immersion in 100% *Gracilaria sp.* extract reduced microbial counts from 2.4×10^4 CFU/g at 4 h to 1.9×10^4 CFU/g at 8 h, remaining within SNI standards. Antimicrobial activity is attributed to secondary metabolites (flavonoids, phenols, saponins, steroids, alkaloids, terpenoids, quinones) that damage bacterial membranes and cytoplasm (28). Flavonoids denature proteins, while phenols disrupt membrane permeability, especially during bacterial cell division (29). *Gracilaria* also contains chloramphenicol-like compounds that inhibit protein synthesis. ANOVA indicated significant effects ($p = 0.001$), with P2, P3, and P+ in the same subset.

Combined Moringa–Seaweed Extract Immersion

The combination treatment yielded counts of 2.5×10^4 at 4 h, 2.2×10^4 at 6 h, and 2.1×10^4 at 8 h, suggesting synergistic effects where Moringa provides bacteriostatic action and *Gracilaria* exerts bactericidal effects. Moringa leaves (*Moringa oleifera*) tend to exhibit bacteriostatic activity, as their active compounds (flavonoids, alkaloids, phenols, and tannins) inhibit bacterial growth and metabolism without directly causing cell death. In contrast, seaweed extracts (*Gracilaria spp.* and related species) demonstrate bactericidal properties, since sulfated polysaccharides, marine phenolics, and halogenated metabolites are capable of disrupting bacterial cell membranes, ultimately leading to cell death. Chloramphenicol-like compounds in seaweed contribute to both actions. Formalin-treated samples showed complete bacterial elimination by 8 h but pose serious health risks upon repeated consumption. Controls experienced continuous quality deterioration due to postmortem biochemical and microbial processes. ANOVA confirmed significant effects ($p = 0.001$), with P2, P3, and P+ not significantly different.

Conclusion

Soaking anchovies in moringa leaf extract, seaweed extract, or their combination significantly preserved organoleptic quality and reduced microbial counts to meet the Indonesian National Standard (SNI), although effectiveness decreased with extended soaking time. Seaweed extract demonstrated more stableantibacterial activity compared to moringa leaf extract, while their combination provided synergistic effects for certain quality parameters. Seaweed extracts are more resistant to environmental degradation, resulting in consistent antibacterial effects. In contrast, the antibacterial activity of moringa leaf extracts tends to be more variable, as they are easily oxidized. Future research should explore optimization of concentration, soaking duration, and the incorporation of other safe natural preservatives as viable alternatives for fish preservation.

Declarations

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Conflict of Interest

The author declares no conflicting interest.

Data Availability

The unpublished data is available upon request to the corresponding author.

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Additional Information

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