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Effects of Combined Matoa and Betel Leaf Extracts as a Natural Preservative on the Quality of Tilapia (*Oreochromis niloticus*)

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ABSTRACT

The use of chemical preservatives, such as formalin, in food products poses significant health risks. Fresh tilapia (Oreochromis niloticus), with a moisture content of 70–80%, is particularly prone to spoilage, underscoring the need for safer, natural preservation methods. This study assessed the quality of tilapia preserved using a combination of matoa (*Pometia pinnata*) and betel leaf (*Piper betle*) extracts. A Completely Randomized Design (CRD) was implemented, featuring five extract treatments (P1–P5), a negative control (K–), and a positive control (K+), each with three replications. Fish samples were immersed in the designated treatment solutions and stored at room temperature for 24 hours, with evaluations conducted at 0, 6, 12, 18, and 24 hours. Parameters measured included organoleptic quality (eyes, gills, mucus, odor, and texture), pH, nitrite, and ammonia levels. Although overall fish quality declined over time, several treatments preserved freshness for up to 18 hours. Compared with K–, the extract-treated groups showed a slower decline in fish quality, while K+ exhibited the greatest stability. Among the extract-treated groups, P3 showed the most favorable overall descriptive performance. These results suggest that combined matoa and betel leaf extracts are effective natural preservatives for extending the shelf life of tilapia during storage.

INTRODUCTION

Indonesia has extensive freshwater resources totaling approximately 18,316,265 km^2, including 17,955,544 km^2 of public waters and 361,099 km^2 of aquaculture waters (Febrian et al., 2022). Of the estimated 7,000 fish species worldwide, approximately 2,000 are found in Indonesia, highlighting the significance of fish in both national consumption and the economy. Fish are recognized for their high nutritional value, particularly their protein content, and are both accessible and affordable. The considerable diversity and broad distribution of fish species contribute significantly to ecosystems and human livelihoods (Syukur et al., 2025). Numerous fish species inhabit Indonesian waters, spanning both marine and freshwater environments. Among freshwater species, tilapia is especially popular and widely consumed (Wicaksono et al., 2025).

Tilapia (*Oreochromis niloticus*) is a freshwater fish species with considerable potential to improve national food security and generate significant economic value, driven by rising domestic consumption and international market demand. In Indonesia, tilapia is among the most prominent aquaculture commodities and continues to experience rising market demand. In addition to meeting local consumption needs, tilapia is exported to the United States

primarily as fillets (Prihatini et al., 2023). The global annual demand for tilapia is estimated at approximately 200,000 tons. Due to its affordability, tilapia is widely consumed for its high protein content and essential amino acid profile, which provide important health benefits (Colli et al., 2026). Nutritionally, tilapia meat contains about 17.7% protein, 1.3% fat, 1–2% minerals, and a moisture content between 70% and 80% (Miska Sanda Lembang, Christian Tandi Arung, 2025).

Fish freshness is at its highest immediately after death; therefore, its quality can only be maintained, not improved, through proper handling. As a highly perishable food product, fish quality begins to decline immediately after capture or death. As a result, fish processing is designed to prevent or slow spoilage and preserve quality during storage (Asni et al., 2022). For instance, tilapia may show physical deterioration within two hours post-mortem (Fransisca & Muhsoni, 2021). Spoilage is primarily driven by enzymatic activity, microbial growth, and oxidation, which result in unpleasant odors, firm flesh, cloudy eyes, and mucus accumulation on the gills and body surface. The high moisture content (70–80%), near-neutral pH, and unsaturated fatty acid composition further promote bacterial proliferation and the development of rancid odors. Consequently, effective preservation methods are necessary to extend the shelf life of fish while retaining its quality and nutritional value (Arifin & Nugroho, 2016).

Fish preservation techniques are employed to extend shelf life; however, the use of chemical substances such as formalin and borax remains prevalent. Formalin is prohibited in food products due to its harmful health effects (R.J et al., 2025). The Regulation of the Minister of Health of the Republic of Indonesia No. 722/MenKes/Per/IX/1988 classifies formalin as a banned food additive because of its adverse effects when present in food products (Yanuari et al., 2025). Despite this regulation, traders frequently misuse formalin to maximize profits and reduce losses from unsold products. Formalin is often selected because it is inexpensive, readily available, and highly effective as a preservative (Nurfadhila et al., 2022).

According to the 2024 inspection results from the Indonesian Food and Drug Authority (BPOM RI), 102 out of 9,262 food samples tested (1.1%) contained prohibited substances, with formalin detected in 0.53% of samples (Sari et al., 2025). The continued use of chemical preservatives in fishery products has been reported in multiple regions. Analysis of salted fish samples at the Laboratory of the Faculty of Mathematics and Natural Sciences, Universitas Bengkulu, indicated that all tested samples contained formalin. Among the 28 samples collected from five locations four in Bengkulu City and one on Enggano Island formalin concentrations in Bengkulu City ranged from 0.04% to 0.26%, while those from Enggano Island ranged from 0.13% to 0.41% (Gazali et al. 2022).

The Regulation of the Minister of Health of the Republic of Indonesia No. 033 of 2012 on food additives classifies borax and formalin as prohibited food additives due to their potential health risks. As a result, the use of hazardous chemicals such as formalin and borax is not considered an appropriate solution. There is a need for safer, healthier, and more environmentally friendly preservative alternatives (Ministry of Health of the Republic of Indonesia, 2012). Plants containing antibacterial compounds, such as Pometia pinnata and Piper betle, represent potential natural preservative alternatives.

Matoa leaves *(Pometia pinnata)* contain a range of secondary metabolites, such as *flavonoids, alkaloids, tannins, saponins, terpenoids, glycosides,* and *coumarins* (Pinontoan et al., 2025). These phytochemical compounds exhibit antibacterial, antifungal, and antioxidant properties. *Flavonoids* inhibit microbial growth by damaging cell membranes, *alkaloids* act as antimicrobial agents, and *tannins* precipitate proteins and compromise bacterial cell walls. Additionally, *saponins* disrupt microbial cell membranes, while *terpenoids, glycosides,* and *coumarins* further contribute to antimicrobial activity, thereby slowing the spoilage process (Sulistijowati et al., 2020).

Betel leaves (*Piper betle)* contain bioactive compounds, including *flavonoids, alkaloids, polyphenols, tannins,* and essential oils, which serve as antibacterial agents. The *polyphenols* and essential oils, such as *eugenol,* inhibit the growth of spoilage bacteria and reduce oxidation in

fish. *Flavonoids* and *alkaloids* disrupt microbial protein synthesis, while *tannins* inhibit bacterial enzyme activity. Essential oils from betel leaves also contain *betelphenol, cineol, caryophyllene,* and *kavikol,* which further enhance antimicrobial properties (Parni et al., 2024). Green and red betel leaf extracts differ in antioxidant capacity. Green betel leaves demonstrate significantly stronger antioxidant activity, with an IC_{50} of 2.0375 µg/mL compared to 50.1187 µg/mL for red betel leaves, indicating greater efficacy in inhibiting oxidation and food spoilage (Zulfah & Amananti, 2021).

Previous studies have demonstrated that betel leaf extract exhibits antibacterial activity, inhibiting the growth of spoilage microorganisms in fish; however, these observations were limited to 6 hours of storage (Rika et al., 2026). In contrast, matoa leaf extract has shown potential as a natural antibacterial and antioxidant agent due to its flavonoid, tannin, and saponin content, which can inhibit microorganisms and slow oxidation during storage for up to 12 hours (Sulistijowati et al., 2020). Given these properties, the combination of betel and matoa leaf extracts is expected to inhibit spoilage microorganisms and delay deterioration in fish quality. Nevertheless, research on the application of *Pometia pinnata* and *Piper betle* leaf extracts as natural preservatives for tilapia remains scarce. This study aims to evaluate the effectiveness of combining these two extracts in maintaining fish quality during storage.

METHOD

The research was conducted from December 2025 to February 2026. The extraction process took place at the Science Laboratory of the Faculty of Teacher Training and Education, Universitas Bengkulu, Bengkulu City. An experimental approach was utilized, employing a one-factor Completely Randomized Design (CRD) to examine variations in the concentration of combined matoa leaf and betel leaf extracts.

Materials included tilapia, matoa leaves, betel leaves, distilled water, 37% *formaldehyde solution* (used as the positive control), ammonia test reagents (NH_3/NH_4^+), and nitrite test reagents (NO_2^-). Young leaves were selected from the top three shoots. Equipment comprised jars, sample bottles, digital scales, knives, cutting boards, blenders, measuring cylinders, filters, a pH meter, tweezers, syringes, basins, gloves, and mobile phones.

In the preparation phase, *Pometia pinnata* and *Piper betle* leaf extracts were prepared separately by processing approximately 562.5 grams of fresh leaves to yield 1,125 mL of extract per species, maintaining a 1:2 (w/v) material-to-extract ratio. The leaves were washed, cut, blended with distilled water, and filtered to produce individual stock extracts. Treatment solutions were formulated by mixing equal volumes of the two stock extracts and diluting with distilled water to a final volume of 500 mL. This process generated concentrations of 10%, 20%, 30%, 40%, and 50%, corresponding to 25, 50, 75, 100, and 125 mL of each extract, respectively, in addition to negative and positive controls.

Treatments included five extract concentration levels, a negative control, and a positive control, each replicated three times. Fresh tilapia, weighing 100–250 grams per fish, were sourced from aquaculture ponds in Bengkulu City. Prior to treatment, internal organs were removed, and the fish were washed with clean water. The prepared tilapia were immersed in the designated treatment solutions and stored at room temperature. Observations were conducted at 0, 6, 12, 18, and 24 hours. Samples were removed from the solutions only during testing.

Fish quality was evaluated using organoleptic, pH, ammonia, and nitrite tests, each conducted in triplicate. The organoleptic assessment utilized the hedonic scale method and involved four panelists, consisting of one semi-trained panelist, two general panelists, and one untrained panelist. The panelists were selected based on their willingness to participate in the organoleptic assessment. pH values were measured using a pH meter, while ammonia and nitrite concentrations were determined with chemical test kits. The resulting data were analyzed statistically to assess the effects of the treatments, supplemented by descriptive analysis. Detailed information regarding the treatment design and solution compositions is provided in Table 1.

Table 1. Research Treatment Design

No	Treatment Code	Treatment Composition	Matoa and Betel Leaf Extract Solution
1.	Negative Control (K−)	500 mL distilled water	-
2.	Positive Control (K+)	10% formalin	50 mL formalin + 450 mL distilled water
3.	P1	10% (combined extract) +90% distilled water	50 mL (25 mL matoa leaf extract + 25 mL betel leaf extract) + 450 mL distilled water = 500 mL
4.	P2	20% (combined extract) +80% distilled water	100 mL (50 mL matoa leaf extract + 50 mL betel leaf extract) + 400 mL distilled water = 500 mL
5.	P3	30% (combined extract) +70% distilled water	150 mL (75 mL matoa leaf extract + 75 mL betel leaf extract) + 350 mL distilled water = 500 mL
6.	P4	40% (combined extract) +60% distilled water	200 mL (100 mL matoa leaf extract + 100 mL betel leaf extract) + 300 mL distilled water = 500 mL
7.	P5	50% (combined extract) +50% distilled water	250 mL (125 mL matoa leaf extract + 125 mL betel leaf extract) + 250 mL distilled water = 500 mL

RESULT AND DISCUSSION

The effectiveness of combined extracts of *Pometia pinnata* and *Piper betle* leaves in preserving tilapia (*Oreochromis niloticus*) was evaluated using both organoleptic and chemical quality parameters. Organoleptic quality was assessed through sensory evaluation, while chemical quality was determined by measuring pH, nitrite, and ammonia levels. The results for each parameter are presented and discussed in the subsequent sections.

Organoleptic Characteristic

The organoleptic test is a sensory evaluation process performed by humans to assess product preference using the five senses (Hanung et al., 2025). Organoleptic quality was determined based on the sensory attributes of eyes, gills, mucus, odor, and texture, following the guidelines outlined in SNI 2729:2013. Figure 1 displays the overall trend of organoleptic quality changes during storage, while Table 2 provides detailed organoleptic scores for each sensory attribute across various treatments.

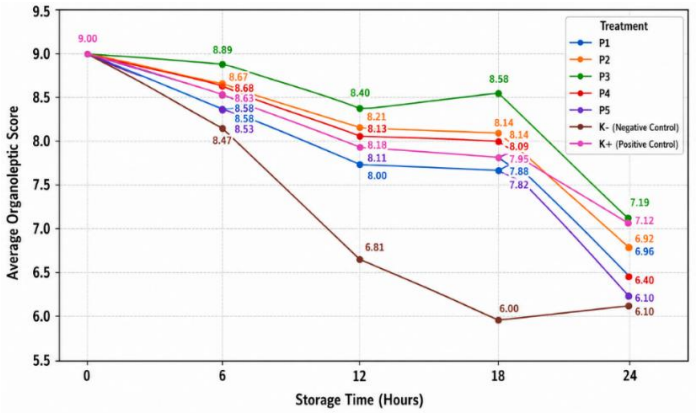


Figure 1. Mean organoleptic scores of tilapia for each treatment throughout the storage period

Table 2. Quality deterioration of tilapia across all treatments (P1–P5, K–, K+) over 24 hours of storage, as measured by average organoleptic scores.

Time/ Hours	Param-eters	Average organoleptic scores from Panelists 1 to 4							Mean	SD	Desc
		P1	P2	P3	P4	P5	K-	K+			
0	Eyes	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	0.00	Fresh
	Gills	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	0.00	Fresh
	Mucus	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	0.00	Fresh
	Odor	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	0.00	Fresh
	Texture	9.00	9.00	9.00	9.00	9.00	9.00	9.00	9.00	0.00	Fresh
6	Eyes	8.58	8.30	8.58	8.66	8.00	8.33	7.83	8.33	0.31	Fresh
	Gills	8.58	8.25	9.00	8.50	8.50	7.75	8.25	8.40	0.38	Fresh
	Mucus	9.00	9.00	9.00	9.00	9.00	8.83	9.00	8.98	0.06	Fresh
	Odor	9.00	9.00	9.00	9.00	9.00	8.98	9.00	9.00	0.01	Fresh
	Texture	8.83	8.92	9.00	9.00	8.83	8.67	9.00	8.89	0.12	Fresh
12	Eyes	7.42	8.00	8.08	7.50	7.83	6.75	7.25	7.55	0.47	Fresh
	Gills	7.75	8.00	8.42	7.83	8.00	7.00	7.50	7.79	0.45	Fresh
	Mucus	8.83	9.00	8.83	8.83	8.92	7.00	9.00	8.63	0.72	Fresh
	Odor	8.25	8.58	8.75	8.67	9.00	6.83	9.00	8.44	0.76	Fresh
	Texture	8.25	8.58	8.75	8.67	9.00	6.83	9.00	8.44	0.76	Fresh
18	Eyes	7.08	7.58	8.33	7.58	7.75	6.25	6.25	7.26	0.78	Fresh
	Gills	7.00	7.50	8.00	7.67	7.92	5.83	6.50	7.20	0.80	Fresh
	Mucus	8.50	8.83	8.83	8.67	7.83	5.92	9.00	8.23	1.09	Fresh
	Odor	8.67	8.83	8.83	8.67	8.58	5.75	9.00	8.33	1.15	Fresh
	Texture	8.67	9.00	9.00	8.83	8.67	6.25	9.00	8.49	1.00	Fresh
24	Eyes	6.33	6.92	7.42	6.75	6.50	4.83	5.17	6.27	0.94	Not Fresh
	Gills	6.25	6.75	7.17	6.08	6.17	5.25	5.58	6.18	0.65	Not Fresh
	Mucus	7.50	7.25	7.17	6.42	6.50	6.42	7.83	7.01	0.57	Fresh
	Odor	6.67	6.67	6.92	6.83	6.08	7.00	9.00	7.02	0.92	Fresh
	Texture	6.75	7.00	7.25	6.00	6.00	7.00	9.00	7.00	1.01	Fresh

Note: The assessment of fish freshness referred to SNI 2729:2013, with criteria of scores ≥ 7.0 indicating fresh/acceptable for consumption and scores < 7.0 indicating not fresh/ unacceptable for consumption.

Figure 1 and Table 2 show a consistent decline in the organoleptic quality of tilapia (*Oreochromis niloticus*) during a 24-hour storage period. Initially, all sensory parameters, eyes, gills, mucus, odor, and texture, received the maximum score of 9.00, reflecting optimal freshness. These scores decreased steadily as storage time increased. The average scores for all sensory parameters remained at or above 7.0 up to 18 hours, meeting the freshness criteria outlined in SNI 2729:2013. By 24 hours, the average scores for eyes (6.27) and gills (6.18) fell below the acceptable threshold, indicating a loss of freshness. Appendix 1 includes representative photographs of the eyes and gills at 24 hours to illustrate these visual changes. In contrast, mucus (7.01), odor (7.02), and texture (7.00) remained within the acceptable range for fresh fish. At 24 hours, P3 showed the highest scores among the extract treatments for eyes (7.42), gills (7.17), and texture (7.25), whereas the highest scores for mucus and odor were obtained by K+ (7.83 and 9.00, respectively). To determine whether the differences in organoleptic scores among P1, P2, P3, P4, P5, K–, and K+ at 24 hours were statistically significant, the Kruskal–Wallis test was performed. The analysis showed a significant difference

for the eyes parameter ($p = 0.029$; $p < 0.05$), whereas the gills ($p = 0.069$), mucus ($p = 0.061$), odor ($p = 0.120$), and texture ($p = 0.062$) parameters did not show significant differences ($p > 0.05$). These findings provide statistical support for the descriptive organoleptic observations and indicate that treatment-related differences were evident only for the eye parameter, while the higher scores observed for certain treatments in the other sensory parameters should be interpreted as descriptive differences rather than statistically significant treatment effects.

The decline in organoleptic quality is attributed to postmortem autolysis and the proliferation of spoilage microorganisms that utilize fish proteins as nutrient sources. These biological processes progressively alter the physical and sensory characteristics of fish, resulting in cloudy eyes, gill discoloration, increased mucus production, loss of the characteristic fresh odor, and reduced muscle firmness (Santoso et al., 2017). These changes intensify with extended storage due to continued muscle protein degradation and the accumulation of microbial metabolites.

These findings are consistent with previous studies indicating that *Pometia pinnata* leaves contain phenolic compounds, flavonoids, tannins, and other bioactive constituents with antimicrobial and antioxidant activities that help maintain fish freshness during storage (Sulistijowati et al., 2020). Similarly, *Piper betle* extract contains *flavonoids, tannins*, and *essential oils* that effectively inhibit the growth of spoilage microorganisms (Rika, Fachria & Anggita, 2026). The synergistic action of these bioactive compounds likely contributed to the delayed deterioration observed in treatment P3, establishing this concentration as optimal for maintaining the organoleptic quality of tilapia during storage.

While increasing extract concentration generally enhanced preservative activity, treatment P5 (50%) demonstrated lower preservation effectiveness than P3. Excessively high concentrations of plant extracts do not necessarily improve preservation efficiency and may exceed the tolerance of fish tissues, leading to adverse effects (Yuniar et al., 2022). At elevated levels, flavonoids may undergo autoxidation, shifting from antioxidant to pro-oxidant activity and accelerating lipid oxidation and rancidity (Kurek et al., 2024). Additionally, excessive tannins and phenolic compounds may interact with muscle proteins, causing protein precipitation and denaturation (Tang et al., 2024). These structural changes reduce the water-holding capacity of fish muscle, resulting in a softer texture and lower sensory acceptance.

In summary, the combination of *Pometia pinnata* and *Piper betle* leaf extracts effectively delayed the deterioration of organoleptic quality during storage, with treatment P3 providing optimal preservation for up to 18 hours. Fish preserved with this treatment remained organoleptically acceptable in accordance with SNI 2729:2013 and could be further processed after appropriate cooking. Previous studies have demonstrated that using herbs and spices during cooking enhances aroma and flavor while reducing undesirable odors in stored fish (Zhang et al., 2023). Therefore, tilapia preserved with treatment P3 may be suitable for processing into spice-based culinary products, such as *ikan bumbu kuning*, *pesmol*, *ikan woku*, and *ikan bakar kecap*, in which aromatic seasonings may further enhance consumer acceptance.

Chemical Test

Chemical testing is a key indicator of fish freshness, reflecting biochemical changes that occur during storage due to enzymatic reactions, protein degradation, and microbial activity. In this study, chemical quality was evaluated by measuring pH, nitrite, and ammonia. These parameters were selected because they represent distinct stages of fish spoilage and provide complementary information regarding the preservative effectiveness of the combined *Pometia pinnata* and *Piper betle* leaf extracts. The results for each chemical parameter are presented and discussed in the following sections.

Ph value

Among the chemical parameters evaluated, pH is a widely recognized indicator of fish freshness because it reflects biochemical changes associated with postmortem metabolism and microbial activity during storage (Soambaton et al., 2024). Fresh fish typically exhibits a near-

neutral pH, which initially decreases as a result of postmortem glycolysis and lactic acid formation. During extended storage, microbial metabolism and protein degradation generate alkaline compounds, particularly ammonia, leading to a gradual increase in pH (Najjah et al., 2023). Table 3 presents the pH values of tilapia subjected to different treatments throughout the storage period.

Table 3. pH values of tilapia (*Oreochromis niloticus*) at each storage interval (Mean ± SD)

Time (hours)	Treatment						
	P1	P2	P3	P4	P5	K-	K+
0	6.17 ± 0.92	5.73 ± 0.57	5.99 ± 0.75	5.85 ± 0.47	5.61 ± 1.26	6.61 ± 0.49	5.46 ± 0.75
6	6.35 ± 0.48	6.29 ± 0.57	6.09 ± 0.57	6.34 ± 0.64	6.03 ± 0.67	6.45 ± 0.55	6.09 ± 0.58
12	6.48 ± 0.64	6.49 ± 0.68	6.34 ± 0.69	6.31 ± 0.85	6.04 ± 0.93	6.46 ± 0.70	6.29 ± 0.73
18	6.62 ± 0.59	6.29 ± 0.47	6.39 ± 0.43	6.25 ± 0.48	6.55 ± 0.27	6.99 ± 0.07	6.61 ± 0.63
24	7.40 ± 0.40^{a}	6.60 ± 0.66^{ab}	6.50 ± 0.52^{bc}	6.43 ± 0.58^{cd}	6.71 ± 0.04^{cd}	7.18 ± 0.16^{bd}	5.90 ± 0.11^{d}

Note: Fresh fish generally have pH values < 7, whereas fish flesh with pH values > 7 is classified as less fresh and undergoing spoilage (Fransisca et al., 2025).

Based on Table 3, the pH values of tilapia (*Oreochromis niloticus*) generally increased with storage time. Most treatments maintained pH values within the acceptable freshness range up to 18 h of storage, whereas at 24 h, treatments P1 and K− exceeded the freshness threshold (pH > 7), indicating quality deterioration. In contrast, treatments P2, P3, P4, P5, and K+ maintained pH values below 7.0 throughout storage. These findings are consistent with the standard reported by Fransisca et al. (2025), which states that fish with pH values above 7 generally undergo spoilage and are no longer suitable for consumption.

Prior to hypothesis testing, data normality was assessed using the Shapiro–Wilk test. Treatments K−, K+, and P5 did not follow a normal distribution ($p < 0.05$), while P1, P2, P3, and P4 were normally distributed ($p > 0.05$). Levene's test indicated homogeneity of variances among treatments ($p = 0.610$). Due to the lack of normality in all groups, the Kruskal–Wallis test was employed to analyze differences among treatments.

The Kruskal–Wallis test identified significant differences among treatments ($\chi^2 = 16.327$, $p = 0.012$), indicating that at least one treatment differed from the others. Bonferroni post hoc comparisons, however, did not reveal any significant pairwise differences ($p > 0.05$). As descriptive analysis showed that P3 and P4 had the lowest mean pH values, a Mann–Whitney U test was conducted to compare these treatments. This test also found no significant difference between P3 and P4 ($U = 98.500$, $Z = -0.581$, $p = 0.561$), indicating that both treatments were similarly effective in maintaining pH stability.

The rise in pH during storage is primarily linked to enzymatic activity and microbial degradation of fish proteins. Fish muscle contains abundant protein, providing nutrients for spoilage microorganisms. During storage, proteins are hydrolyzed into peptides and amino acids, which are then deaminated to produce alkaline compounds, especially ammonia. The accumulation of ammonia elevates the pH of fish muscle, signaling progressive deterioration in quality (Najjah et al., 2023). This process accounts for the gradual increase in pH observed during storage.

Relative to the negative control, treatments incorporating both *Pometia pinnata* and *Piper betle* leaf extracts maintained more stable pH values, demonstrating their capacity to inhibit protein degradation and limit the formation of alkaline compounds. This preservative effect is attributed to the antibacterial properties of flavonoids, tannins, and essential oils found in both extracts, which suppress the growth of spoilage microorganisms (Janna et al., 2026). As a result, the combined extracts effectively preserved pH stability and delayed quality deterioration for up to 18 hours of storage.

Although P4 showed a marginally lower mean pH than P3, this difference was not statistically significant. Consequently, P3 was selected as the optimal treatment based on its

consistently superior performance across all quality indicators, including organoleptic quality, nitrite content, and ammonia content. Collectively, these results demonstrated that P3 provided the best preservation of tilapia quality during storage.

Nitrite (NO_2^-)

Nitrite (NO_2^-), an intermediate compound in the nitrogen cycle produced by nitrifying microorganisms oxidizing ammonia, serves as a widely recognized indicator of fish spoilage because it reflects microbial activity during protein degradation (Sari et al., 2024). In addition to pH, nitrite concentration was measured to assess chemical deterioration in tilapia (*Oreochromis niloticus*) during storage. Representative colorimetric results are presented in Appendix 2, and quantitative nitrite concentrations for treatments P1–P5 are provided in Table 4.

Table 4. Nitrite (NO_2^-) concentrations (mg/L) in fish samples under treatments P1–P5

Time (hours)	Treatment						
	P1	P2	P3	P4	P5	K-	K+
0	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00	0.00 ± 0.00
6	0.03 ± 0.05	0.00 ± 0.00	0.00 ± 0.00	0.05 ± 0.06	0.00 ± 0.00	0.07 ± 0.06	0.00 ± 0.00
12	0.15 ± 0.06	0.13 ± 0.06	0.02 ± 0.04	0.16 ± 0.07	0.05 ± 0.06	0.23 ± 0.07	0.00 ± 0.00
18	0.18 ± 0.08	0.20 ± 0.16	0.05 ± 0.06	0.15 ± 0.08	0.13 ± 0.06	0.38 ± 0.11	0.08 ± 0.10
24	0.35 ± 0.28	0.50 ± 0.26	0.20 ± 0.18	0.44 ± 0.37	0.48 ± 0.72	0,75 ± 0.00	0.18 ± 0.18

Note: Nitrite concentrations below the method detection limit (<0.25 mg/L) were assigned a value equal to half the detection limit (0.125 mg/L).

Table 4 shows that nitrite concentrations increased progressively during storage across all treatments, indicating a gradual decline in fish quality. Nitrite levels were undetectable at 0 hours in all groups and increased over time, reaching maximum values after 24 hours. Among the extract-treated groups (P1–P5), treatment P3 consistently had the lowest nitrite concentration at the end of storage (0.20 ± 0.18 mg/L), while P2 had the highest (0.50 ± 0.26 mg/L). In the control groups, the negative control (K−) exhibited the highest nitrite concentration (0.75 ± 0.00 mg/L) after 24 hours, whereas the positive control (K+) maintained substantially lower nitrite levels (0.18 ± 0.18 mg/L). These results suggest that the combined extracts of *Pometia pinnata* and *Piper betle* inhibited nitrite formation compared to the untreated control, with treatment P3 demonstrating the strongest inhibitory effect among the extract-treated groups.

Before hypothesis testing, nitrite data were evaluated for normality and homogeneity of variance. The Shapiro–Wilk test showed that the data were not normally distributed ($p < 0.05$), and Levene's test indicated that homogeneity of variances was not achieved ($p = 0.002$). Consequently, differences among treatments were analyzed using the non-parametric Kruskal–Wallis test. The results indicated no significant differences in nitrite concentrations among the extract-treated groups ($\chi^2 = 4.527$, df = 4, $p = 0.339$). Although mean nitrite concentrations varied numerically, these differences were not statistically significant, suggesting that all extract treatments were similarly effective at suppressing nitrite formation during storage.

The progressive increase in nitrite concentration is associated with microbial degradation of fish proteins during storage. Fish muscle contains abundant proteins that hydrolyze into peptides and free amino acids as spoilage advances. These amino acids undergo deamination, producing ammonia as the primary nitrogenous by-product. Under aerobic conditions, nitrifying bacteria oxidize ammonia to nitrite, leading to a steady accumulation of nitrite during storage (Karlina et al., 2022). Therefore, elevated nitrite concentrations are a reliable indicator of increased microbial metabolism and the corresponding decline in fish quality.

Relative to the negative control, all extract-treated groups exhibited lower nitrite concentrations, indicating effective inhibition of microbial growth and reduced protein

degradation. This preservative effect is attributed to flavonoids, tannins, and essential oils present in *Pometia pinnata* and *Piper betle*, which demonstrate antibacterial activity against spoilage microorganisms (Harijayanti et al., 2026). By limiting microbial proliferation, these bioactive compounds reduce ammonia production and, consequently, restrict nitrite formation during storage. Among the extract-treated groups, treatment P3 consistently resulted in the lowest nitrite accumulation, suggesting that this formulation offers the most effective natural preservation. While the positive control (K+) showed the lowest overall nitrite concentration due to formalin's preservative action, its use is discouraged because of associated health risks. Thus, the combination of *Pometia pinnata* and *Piper betle* extracts, particularly in treatment P3, emerges as a promising natural alternative for preserving the chemical quality of tilapia during storage.

Because nitrite is produced through the oxidation of ammonia during protein degradation, ammonia concentrations were also measured to provide a more comprehensive evaluation of chemical deterioration in tilapia.

Ammonia ($NH_3 + NH_4^+$)

After nitrite concentration was analyzed, ammonia ($NH_3 + NH_4^+$) was evaluated as the primary nitrogenous compound produced during protein degradation and as a precursor to nitrite formation through nitrification. Ammonia is recognized as a key indicator of fish spoilage because it is generated by microbial activity and endogenous autolytic enzymes during storage (Hernanda et al., 2023). As a principal component of Total Volatile Base Nitrogen (TVB-N), ammonia is produced through the deamination of amino acids following fish death. Therefore, an increase in ammonia concentration indicates progressive deterioration of fish quality (Demalinda et al., 2024). Representative results of the colorimetric ammonia analysis are provided in Appendix 3, and the quantitative ammonia concentrations for treatments P1–P5 are shown in Table 5.

Table 5. Ammonia ($NH_3 + NH_4^+$) concentrations in fish samples (mg/L) for treatments P1–P5.

Time (hours)	Treatment						
	P1	P2	P3	P4	P5	K-	K+
0	0.08 ± 0.13	0.00 ± 0.00	0.00 ± 0.00	0.04 ± 0.04	0.00 ± 0.00	0.07 ± 0.08	0.00 ± 0.00
6	0.15 ± 0.19	0.05 ± 0.10	0.12 ± 0.21	0.12 ± 0.10	0.17 ± 0.26	0.35 ± 0.45	0.00 ± 0.00
12	0.23 ± 0.16	0.16 ± 0.10	0.09 ± 0.08	0.23 ± 0.16	0.23 ± 0.30	0.31 ± 0.13	0.03 ± 0.02
18	0.22 ± 0.22	0.26 ± 0.21	0.15 ± 0.11	0.30 ± 0.17	0.44 ± 0.44	0.63 ± 0.21	0.04 ± 0.04
24	0.50 ± 0.27	0.43 ± 0.32	0.29 ± 0.10	0.54 ± 0.25	0.75 ± 0.27	0.83 ± 0.29	0.09 ± 0.06

Note: For calculating mean values, ammonia concentrations below the method detection limit (<0.15 mg/L) were assigned half the detection limit (0.075 mg/L).

Table 5 shows that ammonia concentrations increased progressively throughout the storage period in all treatments, indicating ongoing protein degradation. Among the extract-treated groups (P1–P5), treatment P3 consistently exhibited the lowest ammonia concentration at 24 h (0.29 ± 0.10 mg/L), while P5 had the highest concentration (0.75 ± 0.27 mg/L). In the control groups, the negative control (K–) recorded the highest ammonia concentration (0.83 ± 0.29 mg/L) after 24 h, whereas the positive control (K+) maintained the lowest concentration (0.09 ± 0.06 mg/L) throughout storage. These findings suggest that the combination of *Pometia pinnata* and *Piper betle* leaf extracts reduced ammonia accumulation compared with the untreated control, with treatment P3 demonstrating the greatest inhibitory effect among the extract-treated groups.

Prior to hypothesis testing, ammonia data were assessed for normality and homogeneity of variance. The Shapiro–Wilk test indicated a non-normal distribution ($p < 0.05$), and Levene's test confirmed that the assumption of homogeneity of variances was not met ($p = 0.000$). Consequently, differences among treatments were analyzed using the non-parametric Kruskal–Wallis test. The analysis revealed no statistically significant differences in ammonia

concentrations among the extract-treated groups (χ^2 = 4.150, df = 4, p = 0.386). While descriptive differences in ammonia concentrations were observed, statistical analysis showed that the extract treatments were equally effective in suppressing ammonia formation during storage.

The gradual increase in ammonia concentration during storage is closely linked to microbial metabolism and endogenous proteolytic enzyme activity. After fish death, endogenous enzymes hydrolyze muscle proteins into peptides and amino acids. Spoilage microorganisms then deaminate these amino acids, producing ammonia, a major volatile basic compound (Hernanda et al., 2023; Demalinda et al., 2024). Prolonged storage thus promotes ammonia accumulation as microbial populations expand and protein degradation intensifies. Increasing ammonia concentrations therefore indicate heightened microbial activity and progressive deterioration of fish quality.

Compared with the negative control, treatments containing combinations of *Pometia pinnata* and *Piper betle* leaf extracts maintained lower ammonia concentrations, demonstrating their capacity to inhibit microbial growth and suppress protein degradation. This preservative effect is attributed to the antibacterial properties of bioactive compounds, including flavonoids, tannins, and essential oils, which inhibit the proliferation of spoilage microorganisms, thereby reducing ammonia production (Harijayanti et al., 2026). Among the extract-treated groups, treatment P3 consistently exhibited the lowest ammonia concentration, suggesting a greater ability to delay protein degradation. However, as the Kruskal–Wallis analysis revealed no statistically significant differences among treatments, this finding should be interpreted descriptively rather than as evidence of statistically superior preservative performance.

The negative control exhibited substantially higher ammonia concentrations than all extract-treated groups, indicating unrestricted microbial activity and protein degradation in the absence of preservatives. In contrast, the positive control maintained the lowest ammonia concentrations due to formalin's antimicrobial activity. Although formalin demonstrated the greatest inhibitory effect, its use as a food preservative is not recommended because of its adverse effects on human health. Consequently, the combination of *Pometia pinnata* and *Piper betle* leaf extracts offers a safer natural alternative for reducing ammonia formation during tilapia storage.

The increase in ammonia concentration observed in this study is closely associated with the formation of volatile basic nitrogen compounds, collectively referred to as Total Volatile Base Nitrogen (TVB-N), a widely recognized indicator of fish freshness. Fresh fish typically exhibit TVB-N values below 20 mg N/100 g; values between 20 and 30 mg N/100 g indicate the onset of quality deterioration, while values above 30 mg N/100 g are generally considered unacceptable for consumption (Shafa et al., 2025). Although ammonia concentrations in this study were measured in mg/L and cannot be directly compared with TVB-N values, the relatively low ammonia concentrations (<1 mg/L) observed throughout storage suggest that the samples remained in the early stages of quality deterioration and had not reached severe spoilage. Therefore, ammonia concentration may serve as an early indicator of freshness loss during tilapia storage.

Overall, the progressive increase in ammonia concentration reflected the ongoing deterioration of fish quality during storage. However, the lower ammonia concentrations observed in the extract-treated groups, particularly treatment P3, suggest that the combination of *Pometia pinnata* and *Piper betle* leaf extracts can retard ammonia accumulation by suppressing microbial activity and slowing protein degradation. These findings, along with the results of the pH, organoleptic, and nitrite analyses, indicate that the combined plant extracts are a promising natural preservative for maintaining the chemical quality of tilapia during storage.

CONCLUSION

The results of this study indicate that the combination of *Pometia pinnata* and *Piper betle* leaf extracts has potential as a natural preservative for maintaining both sensory and

chemical quality of tilapia (*Oreochromis niloticus*) during storage. Among the treatments evaluated, P3 was identified as optimal, exhibiting superior performance in preserving organoleptic quality and maintaining favorable pH, nitrite, and ammonia levels. With this treatment, tilapia remained within the acceptable quality range for up to 18 hours of storage.

The findings further demonstrate that increasing the concentration of plant extracts does not necessarily enhance preservative performance. The higher extract concentration applied in treatment P5 reduced preservation effectiveness, indicating that an appropriate balance of bioactive compounds is essential for optimal synergistic activity. These results underscore that preservation efficacy depends on both extract concentration and the interactions among bioactive constituents.

Although the preservative performance of the combined extracts was lower than that of the positive control containing a synthetic preservative, the combination of *Pometia pinnata* and *Piper betle* leaf extracts offers a safer and more environmentally friendly alternative for fish preservation. Further research involving microbiological analyses, extended storage periods, and identification of active phytochemicals is recommended to clarify the preservation mechanism and support practical application in the fish processing industry.

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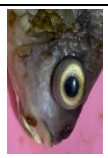
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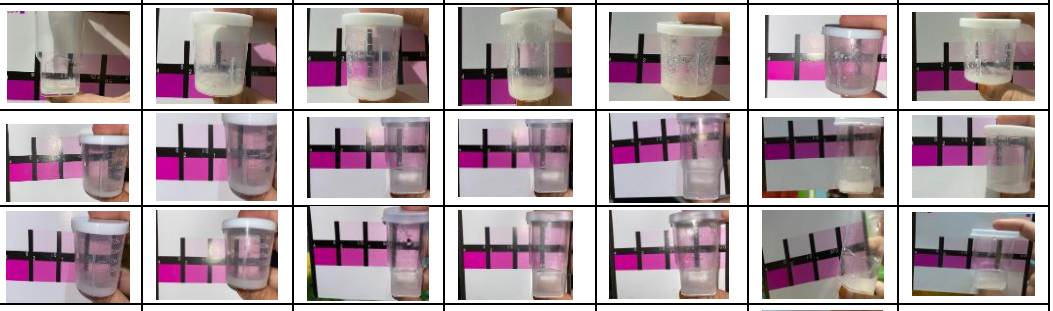
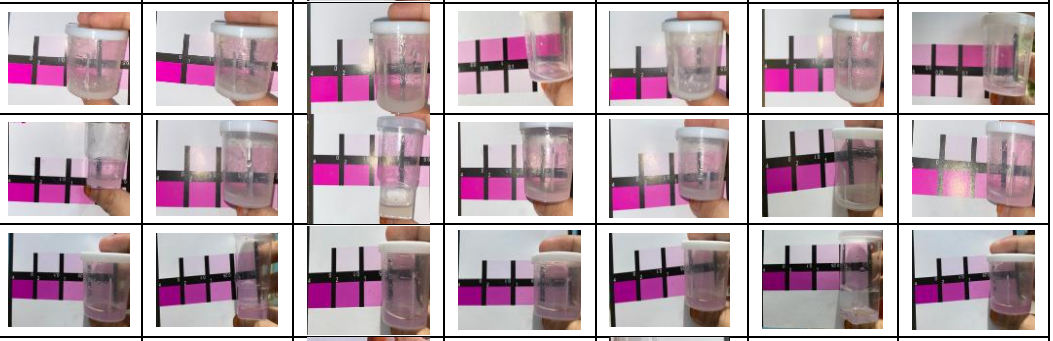
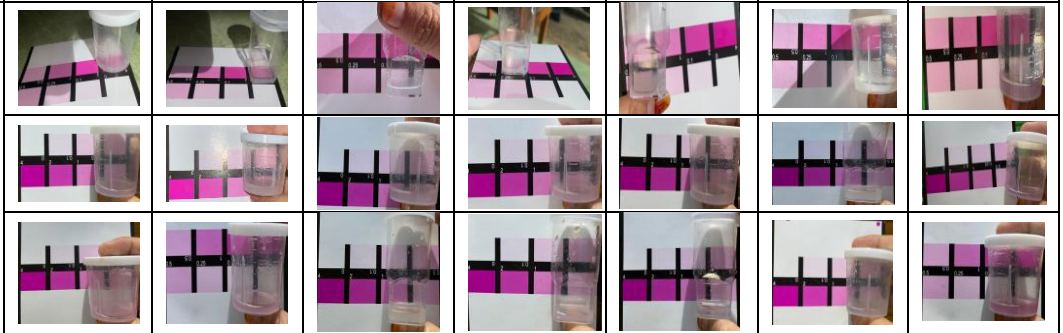
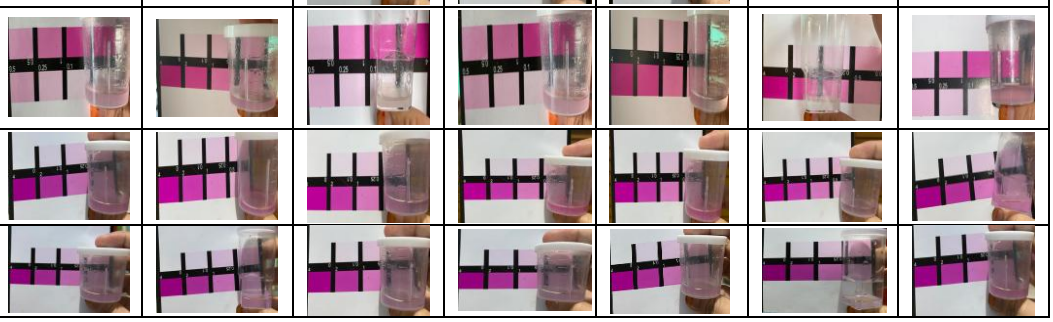
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APPENDIX

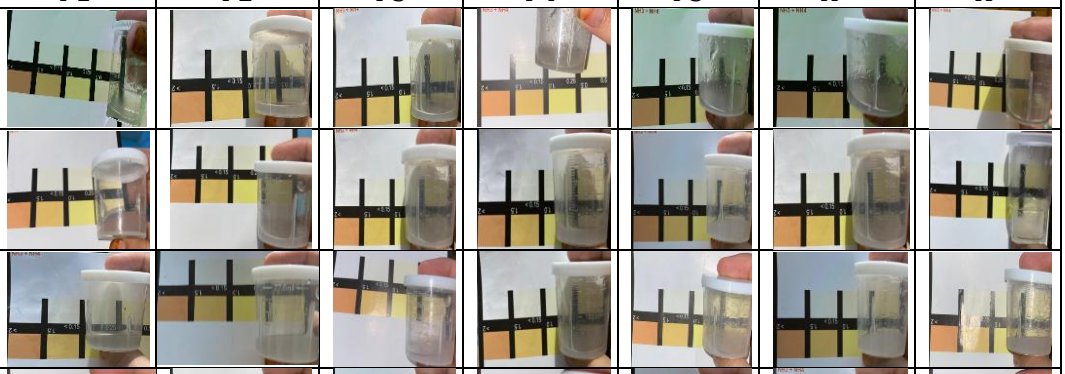
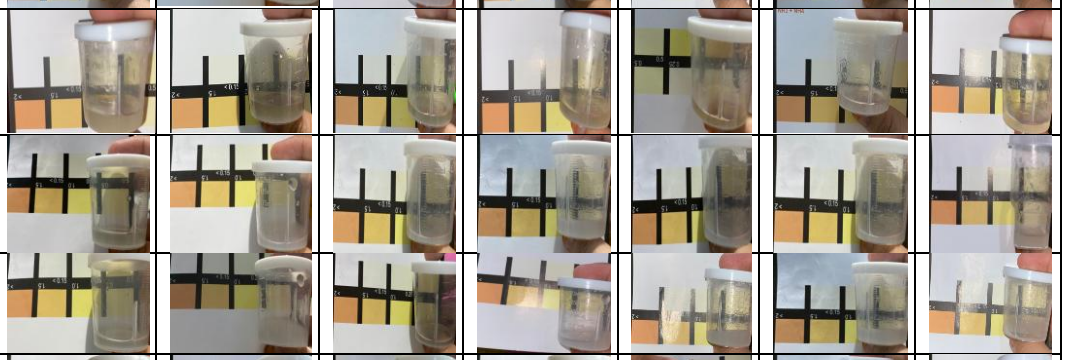
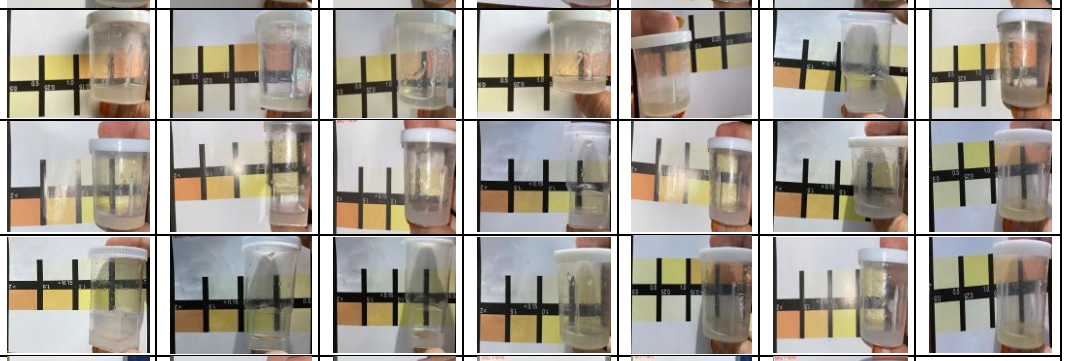
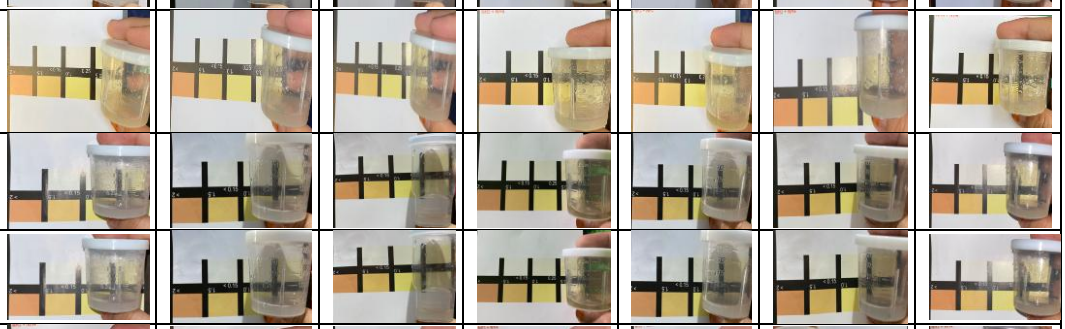
A. Appendix 1

Param-eters	Observed Condition (24 hours)							Fresh Fish Reference
	P1	P2	P3	P4	P5	K+	K-	
Eyes								 Sumber: Jasindo
Gills								 Sumber: Pngtree

B. Appendix 2

Time/ Hours	Average Score from Panelists 1-4						
	P1	P2	P3	P4	P5	K+	K-
0							
6							
12							
18							
24							

C. Appendix 3

Time/ Hours	Figure Ammonia colorimetric test results.						
	P1	P2	P3	P4	P5	K+	K-
0							
6							
12							
18							
24	