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Effectiveness of Mulberry Fruit Infusa (*Morus Alba* L.) as a Natural Preservative for Carp (*Cyprinus carpio*)

I. Srinati [1], Y.A. Septiati [2], F.A. Ayuningtias [1], S. Aripin [2*]

1. Integrated Laboratory Center, Bandung Health Polytechnic, Ministry of Health, Republic of Indonesia
2. Department of Environmental Health, Bandung Health Polytechnic, Ministry of Health, Republic of Indonesia

HIGHLIGHTS

- A 15% extract concentration best maintained carp quality over a 36-hour room temperature storage period.
- TVB-N and protein levels remained within safe limits at 15% extract treatment.
- Bacterial growth was significantly inhibited at 15% and 35% extract concentrations.

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Abbreviations
CFU=Colony Forming Unit
BSN= National Standardization Agency of Indonesia
PCA=Plate Count Agar
SNI=Indonesian National Standard
TPC=Total Plate Count
TVB-N=Total Volatile Base Nitrogen

ABSTRACT

Background: Natural preservatives have been increasingly explored to replace synthetic ones due to their potential health risks. Red mulberry (*Morus alba* L.), rich in phenolic and antimicrobial compounds, served as an effective preservative for freshwater fish. Carp (*Cyprinus carpio*) is widely consumed in Indonesia and highly perishable under room temperature conditions.

Methods: This study was conducted in July 2024 using a completely randomized design. A total of 24 carp samples were divided into four treatment groups (0%, 15%, 25%, and 35% mulberry extract) with six replicates each and stored for 12, 24, and 36 h at room temperature. The mulberry extract was prepared using an infusion method with aquadest in a 1:1 ratio. The quality of carp was evaluated through organoleptic testing with 9-point hedonic scale, Total Volatile Base Nitrogen (TVB-N), protein level with Biuret method, pH test, and total microbial count using the Total Plate Count (TPC) method. The Kolmogorov–Smirnov test was applied due to the non-normal distribution of the data (p<0.005), and further analysis was conducted using a post-hoc test in IBM SPSS version 26.

Results: The 15% extract concentration was the most effective in preserving the quality of carp across all parameters. After 36 h of storage, the pH remained stable at 6.62, the total bacterial count was 2.3×10□ Colony Forming Unit (CFU)/g, and the organoleptic score was maintained at 5. Total Volatile Base Nitrogen (TVB-N) values ranged from 6.72 to 17.93 mg N/100 g, and protein content remained between 15.5% and 17.8%, all within acceptable limits. Microbial growth and pH fluctuations were also better controlled compared to the other treatment groups.

Conclusion: Statistical results confirmed that immersion of carp in 15% red mulberry fruit extract effectively preserved freshness, nutritional quality, and microbiological safety of the fish during 36 h of room temperature storage.

[*] Corresponding author (S. Aripin)
Email: salma.aripin@gmail.com
Orchid ID: https://orcid.org/0000-0002-8627-1623

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Introduction

Artificial preservatives such as benzoic acid (0–5 mg/kg), nitrites and nitrates (0–3.7 mg/kg), and sulfites (0–0.7 mg/kg) are widely used in processed foods. However, excessive consumption has been linked to kidney damage, the formation of carcinogenic nitrosamines, and allergic reactions (World Health Organization [WHO], 2000; Nuwairy, 2015; Rot et al., 2025; Arellano et al., 2011). In Indonesia, misuse of formalin in meat and fish products has also been reported, posing serious risks to public health (Harmita, 2006). These issues highlight the urgent need for safe, natural, plant-based preservatives as alternatives to synthetic compounds.

Food spoilage represents a major challenge to food safety and quality. It occurs when undesirable physical, chemical, or sensory changes—such as odor, texture, or color alterations—reduce food acceptability. While spoilage can result from mechanical or chemical factors, microbial activity is the primary cause. Microorganisms, including molds, yeasts, and bacteria, degrade food macromolecules and release toxins that compromise health (Muchtadi and Ayustaningwarno, 2010). Perishable commodities like meat, milk, eggs, and fish are particularly vulnerable due to their nutrient-rich and high-moisture composition. In meat, spoilage is characterized by slime and odor once microbial counts reach 10^6–10^8 cells/cm² (Koswara, 2009). Similarly, fish spoilage is driven by putrefactive bacteria that produce ammonia, hydrogen sulfide, and other malodorous compounds, leading to discoloration, rancidity, and loss of texture (Koswara, 2009; Muchtadi and Ayustaningwarno, 2010).

To prevent such deterioration, preservatives are commonly applied to inhibit microbial growth and extend shelf life. Synthetic preservatives such as benzoates, nitrates, sorbates, sulfites, and hydrogen peroxide act through various mechanisms, including lowering cytoplasmic pH, disrupting enzyme activity, and interfering with metabolic pathways (Davidson et al., 2005; Davidson et al., 2020). Despite their effectiveness, concerns about toxicity, microbial resistance, and consumer safety have limited their acceptance. Consequently, attention has shifted toward natural antimicrobial compounds. Plant-derived metabolites such as flavonoids, tannins, saponins, glycosides, steroids, and essential oils exhibit both antioxidant and antibacterial properties. Their mechanisms include disrupting cell membrane permeability, inhibiting ribosomal protein synthesis, and interfering with enzyme systems (Carroll et al., 2016). Traditional food practices also demonstrate the preservative potential of natural materials, such as garlic, salt, kluwak, cinnamon, and gambir leaves (Amir et al., 2021; Hayati, 2009).

Among natural resources, mulberry (*Morus spp.*) has attracted attention due to its bioactive components. The genus comprises 19 species, including red mulberry (*Morus alba* L.), which is native to China but widely cultivated in tropical regions like Indonesia. Mulberry fruit is rich in quercetin, anthocyanins, phenolics, and terpenoids, compounds known for antibacterial activity. Previous studies reported that mulberry fruit extract inhibits *Staphylococcus aureus* and *Shigella dysenteriae* (Hastuti et al., 2012), and effectively preserved *Selaroides leptolepis* fish at 30% concentration for 18 h at room temperature (Nastiti et al., 2019). These findings suggest mulberry fruit as a promising candidate for natural food preservation.

Carp (*Cyprinus carpio*), a freshwater fish widely consumed in Indonesia, holds high economic and nutritional value. In 2018, national consumption reached 14.5 billion rupiah, with West Java recording the highest demand (BPS-Statistics Indonesia, 2018). Carp provides approximately 16 g protein per 100 g, along with essential minerals, vitamins, and low mercury levels, making it a valuable protein source (Andra Farm, 2019). However, like other fish, carp is highly perishable and requires preservation strategies to maintain its quality.

Although mulberry extract has demonstrated preservative effects on marine species, its application to freshwater fish such as carp remains underexplored. Moreover, the comparative effects of different extract concentrations have not been thoroughly evaluated. Therefore, this study investigates the preservative effectiveness of red mulberry fruit infusion at 15%, 25%, and 35% concentrations in maintaining the freshness, nutritional quality, and microbiological safety of carp stored at ambient temperature for 36 h.

Material and methods

Materials

Chemicals and reagents used were sterile distilled water (aquadest); Wagner's reagent — potassium iodide (cat. 1.05044, Merck, Germany) and iodine (cat. 1.09099, Merck, Germany); hydrochloric acid (HCl) (cat. 1.00317.2500, Merck, Germany); magnesium powder (cat. 105815, Merck, Germany); Liebermann–Burchard reagents — acetic anhydride (cat. 1.00042, Merck, Germany) and concentrated sulfuric acid (cat. 1.60313, Merck, Germany); ferric chloride (cat. 1.03943, Merck, Germany); 7% trichloroacetic acid (TCA) (cat. 1.00807.0250, Merck, Germany); boric acid (cat. 203667, Sigma-Aldrich, Germany); Tashiro indicator (Methyl Red + Methylene Blue) (cat. 60-016-37, Fisher Scientific, USA); potassium carbonate (K□CO□) (cat. 1.06683, Merck, Germany); BSA (cat. D43294, Merck, Germany); Biuret reagent (cat. 1.10307, Merck, Germany); Plate Count Agar (PCA,

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CM0325B, Oxoid, Swiss).

Sample collection and sampling method

This study was conducted using Completely Randomized Design (CRD) method in which the sample was taken with six repetitions for four treatments (Notoatmodjo, 2010). The population in this study was carp (*C. carpio*). It was purchased in the same fish shop in Bandung traditional market. Sample handling was performed aseptically to avoid contamination. The samples were stored in sterile polypropylene plastic containers at temperatures of maximum 4 °C. When the samples were sent to the test laboratory, they were stored in a cool box containing ice packs and ice gel to maintain the sample temperature.

The independent variable was the extract concentration (15%, 25%, and 35%), while the dependent variables included several quality parameters: organoleptic score, Total Volatile Base Nitrogen (TVB-N), and protein level. Complementary parameters such as pH and Total Plate Count (TPC) were also evaluated.

The Gomez formula was used to determine the number of replications in each treatment: $(t–1)(r–1)\geq15$, where t refers to the number of treatments and r refers to the number of repetitions (Gomez, 2007). There were four treatments in this study, namely the use of extract at concentrations of 0% as the control, 15%, 25%, and 35%. Therefore, the calculation of the number of sample replications is as follows:

$(4 - 1)(r - 1)\geq15$
$3(r - 1)\geq15$
$3r - 3\geq15$
$3r\geq18$
$r\geq6$

Based on the calculation, a minimum of six replications was required per group. Thus, 24 fish were used in total. The experiment was conducted in the Integrated Laboratory of the Polytechnic of Health, Ministry of Health, Bandung, Indonesia, from June to July 2024.

Extraction method

Infusion method was done to make the mulberry fruit extract using aquadest sterilized water with the ratio of weight and volume 1:1 (Bainiwal et al, 2013). Red mulberry fruit was cleaned then weighed 1 kg to be mashed or blended. Following that, the pureed red mulberry fruit was dissolved in 1 L of aquadest. The extraction or immersion was conducted for three days in a brown or dark container, and was stored in a dark room, not exposed to lamp or sunlight. Afterward, the maceration result was filtered to separate the filtrate and residue to be heated on a 90 ^{o}C hot plate for 15 min, stirring occasionally. To get the desired volume, it was then filtered through the flannel and given the right amount of hot water to the residue (Jegabun, 2023).

Analytical methods

The organoleptic test was conducted using a questionnaire sheet based on the Indonesian National Standard (SNI) for sensory testing of fresh fish, which includes assessments of eye appearance, gills, surface mucus, flesh, odor, and texture of the fish, using a 9-point scoring scale. Other chemical materials used for the analysis of carp included those for the TVB test, namely 7% Trichloroacetic acid (TCA) (1.00807.0250, Merck, Germany), Boric acid (203667, Sigma-Aldrich, Germany), Tashiro indicator composed of Methyl Red and Methylene Blue (60-016-37, Fisher Scientific, USA), Potassium carbonate (K_2CO_3) (1.06683, Merck, Germany), and Hydrochloric acid (HCl) (1.00317.2500, Merck, Germany). The organoleptic test was conducted using a questionnaire sheet based on the SNI for sensory testing of fresh fish, which includes assessments of eye appearance, gills, surface mucus, flesh, odor, and texture of the fish, using a 9-point scoring scale. Materials used for protein content analysis included BSA (Bovine Serum Albumin) (D43294, Merck, Germany) and Biuret reagent (1.10307, Merck, Germany).

The analytical equipment used in this study included test tubes and dropper pipettes for phytochemical testing, a hot plate, a 1 L beaker glass, and a water thermometer for extract preparation. The pH testing instrument used was a pH meter (51302803, Mettler Toledo, Switzerland). The titration equipment for TVB analysis included a 25 ml burette (40BC-25, Pyrex, USA) and a Conway unit. The equipment used for protein content analysis included a 25 ml volumetric flask, volumetric pipettes, and a Ultraviolet–visible spectrophotometer (206-55401-92, Shimadzu UV-1700, Japan). For the PCA test, the tools used were volumetric pipettes (4642090, Thermo, USA), a Bio Safety Cabinet (1389-G, Thermo, USA), an autoclave (4814001000, Hirayama, Japan), an incubator (Memmert BE400, Germany), and a colony counter (BCC-116, B-one, Indonesia).

Carp analysis

A carp (*C. carpio*), which was not immersed in the mulberry fruit (*Morus alba* L.) extract, acted as a control carp to be compared with the carp immersed in the mulberry fruit (*Morus alba* L.) extract with variation of 15%, 25%, and 35% concentration for 30 min. The carp were immersed completely in one L of each extract solution for 12, 24, and 36 h at room temperature.

The laboratory tests conducted in this study were organoleptic test in accordance with SNI number 2354.8:2009 (National Standardization Agency of

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Indonesia [BSN], 2013), TVB test in accordance with SNI number 2354.8:2009 (BSN, 2009), protein level test using Biuret method (Scopes, 1987). pH test using pH probe Mettler Toledo type Seven Easy (51302803, Mettler Toledo SevenEasy, Swiss), and TPC bacterial test in accordance with SNI number 01-2332.3-2006 (BSN, 2006).

Phytochemical test result

Phytochemical test is a method used to identify the content of secondary metabolite compounds of natural materials. Qualitative Phytochemical test could be done by activating the extract with a specific reactor to obtain the color reaction (Harborne, 1998).

Organoleptic test

The organoleptic quality of carp was evaluated based on visual and sensory parameters, including eye appearance, gills, surface mucus, flesh, odor, and texture of the fish. The assessment followed the SNI 2354.8:2009 using a 9-point hedonic scale, where higher scores indicate fresher fish. Six trained panelists conducted the evaluation. This test aimed to determine the freshness and consumer acceptability of the fish during storage.

TVB-N test

TVB-N was analyzed using the microdiffusion method in accordance with SNI 2354.8:2009. This test measures the concentration of nitrogenous compounds—such as ammonia, dimethylamine, and trimethylamine—that are produced during microbial and enzymatic protein degradation. The values were expressed in milligrams of nitrogen per 100 grams of fish. This test was used to indicate the degree of spoilage in fish samples.

Protein level test

Protein content was measured using the Biuret method, which detects peptide bonds that form violet-colored complexes in the presence of copper (II) ions under alkaline conditions. Absorbance was read at 540 nm using a Shimadzu Ultraviolet–visible 1700 spectrophotometer. This method is based on Scopes (1987). The protein test was conducted to assess the extent of protein degradation and to evaluate how well the extract preserved the nutritional quality of the fish.

pH test

pH was measured using a Mettler Toledo SevenEasy digital pH meter (51302803, Mettler Toledo, Switzerland). Fish samples were homogenized with distilled water at a 1:10 ratio, and the pH value was recorded once stabilized. Monitoring pH is important because postmortem biochemical processes and microbial activity can alter the acidity of the fish, with increasing pH often indicating spoilage. This test helped determine the stability and freshness of the fish during storage.

TPC test

The microbial load was assessed using the pour plate method based on SNI 01-2332.3-2006, about Microbiological Test Method-Part 3: The Determination of TPC in Fish Product. Samples were serially diluted, plated on PCA (CM0325B, Oxoid, Swiss), and incubated at 35 °C for 48 h. The resulting CFU were counted and expressed in CFU per gram of fish. This test was used to measure bacterial growth over time and to evaluate the antimicrobial effectiveness of the mulberry extract treatments.

Statistical analysis

This study was conducted using Completely Randomized Design (CRD) method in which the sample was taken with six repetitions for four treatments. Data normality was tested using the Kolmogorov–Smirnov test to evaluate differences among groups, since the data were not normally distributed ($p<0.05$). Kruskal Wallis analyses were performed using IBM SPSS Statistics v26.0 (IBM Corp., Armonk, NY, USA), with significance set at $\alpha=0.05$.

Quality control

This study used a method in accordance with the SNI, in which the sample testing process is explained starting from the test principle, the tools and materials needed, sample preparation, test procedures, how to read or calculate samples, and sample quality control. SNAs are technical standards for ensuring the quality of products in Indonesia, which also refer to international standards.

Results and discussion

The carp (C. carpio) sample environmental condition

The environmental condition was also observed when the carp was tested because it affected the test result. It was measured by using a portable thermo hydrometer and the result could be seen in table 1.

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Table 1: The carp (*Cyprinus carpio*) sample environmental condition

No.	The Extract Immersion	The Environmental Condition	
		Temperature (°C)	Humidity (%)
1	0% Control, 12 H Storage	24.8	86
2	0% Control, 24 H Storage	21.3	54
3	0% Control, 36 H Storage	27.4	69
4	15% Concentration, 12 H Storage	25.4	71
5	15% Concentration,24 H Storage	27.3	65
6	15% Concentration, 36 H Storage	25.4	71
7	25% Concentration, 12 H Storage	26.8	71
8	25% Concentration, 24 H Storage	26.7	69
9	25% Concentration, 36 H Storage	27.2	70
10	35% Concentration, 12 H Storage	24.1	61
11	35% Concentration, 24 H Storage	23.8	59
12	35% Concentration, 36 H Storage	27.1	68
	Average	25.92	66.7
	Max	27.8	86
	Min	21.3	54

Environmental conditions were monitored because they influence preservation outcomes. Temperature ranged from 21.3 °C to 27.8 °C and humidity from 54% to 86% (Table 1). Sampling times were 12, 24, and 36 h. The 12 h and 36 h observations were conducted at night, whereas the 24 h observation occurred in the morning. These natural fluctuations may have affected microbial growth and protein degradation.

The result on the carp (*C. carpio*), which was immersed in the various concentration of red mulberry fruit (*Morus alba* L.) extract, could be seen in Table 2.

Table 2: The pH, TPC, TVB-N and protein result of carp immersed in various concentration of red Mulberry (*Morus alba* L.) extract

No	Extract Treatment	Average pH Results	TPC	TVB-N	Protein
1	0% Control, 12 h of Storage	6.39 ^{a}	$1.9x10^{5a}$	13.45 ^{ab}	16.60 ^{a}
2	0% Control, 24 h of Storage	6.49 ^{a}	$4.3x10^{5a}$	20.17 ^{ab}	15.80 ^{a}
3	0% Control, 36 h of Storage	6.76 ^{a}	$5.0x10^{5a}$	29.13 ^{ab}	13.80 ^{a}
4	15% Concentration, 12 h of Storage	6.46 ^{a}	$1.8x10^{5a}$	6.72 ^{b}	17.80 ^{a}
5	15% Concentration, 24 h of Storage	6.59 ^{a}	$2.1x10^{5a}$	13.45 ^{b}	16.40 ^{a}
6	15% Concentration, 36 h of Storage	6.62 ^{a}	$2.3x10^{5a}$	17.93 ^{b}	15.50 ^{a}
7	25% Concentration, 12 h of Storage	6.36 ^{b}	$1.8x10^{5a}$	10.09 ^{a}	12.40 ^{b}
8	25% Concentration, 24 h of Storage	6.89 ^{b}	$3.2x10^{5a}$	36.98 ^{a}	10.50 ^{b}
9	25% Concentration, 36 h of Storage	6.91 ^{b}	$3.3x10^{5a}$	43.7 ^{a}	7.00 ^{b}
10	35% Concentration, 12 h of Storage	5.86 ^{a}	$1.4x10^{4a}$	6.72 ^{ab}	6.50 ^{b}
11	35% Concentration, 24 h of Storage	6.57 ^{a}	$1.9x10^{4a}$	11.21 ^{ab}	5.10 ^{b}
12	35% Concentration, 36 h of Storage	6.76 ^{a}	$3.6x10^{4a}$	41.46 ^{ab}	2.40 ^{b}

Unit for TPC Value: Colony Forming Unit (CFU)/g
Kruskal-Wallis test was used as a statistic test. Different letters in each column indicate statistically significant differences (p<0.05).
TPC=Total Plate Count; TVB-N= Total Volatile Base Nitrogen

The pH, TPC, TVB-N, and protein results of carp immersed in various concentrations of red mulberry (*Morus alba* L.) extract are shown in Table 2. In this study, pH and TPC were evaluated descriptively rather than statistically. These parameters exhibited minimal variation across treatments, and the available dataset did not meet the assumptions required for valid statistical testing. As a result, both pH and TPC are presented narratively to accurately reflect their trends during storage without over interpreting the data.

pH result

pH values increased with storage duration, though differences between treated and control fish were small. Mulberry extract initially appeared to reduce pH by shifting conditions toward acidity, consistent with the extract's inherent pH of 3.25 and its polyphenolic content (Chang et al., 2021). Fluctuations at 24 h and 36 h may reflect may reflect a reduction in antimicrobial effectiveness due to microbial type, age, and concentration (Nastiti et al., 2019). Fresh fish typically has a pH of 6.4–6.6, decreasing after death due to autolysis (Sulistijowati et al., 2020). Previous findings also reported pH reduction in mulberry-treated fish (Nastiti et al., 2019).

TPC result

TPC increased with storage time, but mulberry-treated samples generally showed lower microbial loads than the control, especially at 15% concentration. At 25%, antimicrobial activity declined, likely influenced by extract quality factors such as extraction method, temperature, humidity, and solvent (Marto, 2021). The 35%

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concentration again appeared to reduce TPC. The 15% concentration at 36 h produced 2.3×10^5 CFU/g, below the SNI limit of 5×10^5 CFU/g (BSN, 2013). Mulberry extract contains phenolics effective against bacteria including *Pseudomonas aeruginosa*, *Aspergillus niger*, *S. aureus*, *Eschefrichia coli*, *Saccharomyces cerevisiae*, and *Bacillus subtilis* (Batiha et al., 2023). Mulberry-treated scad fish also appeared to reduce bacterial growth in prior work (Nastiti et al., 2019).

The antimicrobial action of mulberry extract is linked to flavonoids and phenolics, which disrupt permeability, enzymes, genetic material, and cell wall integrity (Liu et al., 2025; Lobiuc et al., 2023). The extract contains alkaloids, saponins, flavonoids, phenolics, and tannins, each contributing antimicrobial effects (Siddiqui et al., 2009). Ethanol extract exhibits stronger antimicrobial activity than water extract (Chang et al., 2021).

TVB-N result

The Kruskal-Wallis test revealed that extract concentration had a significant effect on TVB-N value (p=0.006). The 15% mulberry extract had significantly lower TVB-N than the 25% treatment, while the control and 35% concentrations showed intermediate values. TVB-N increased with storage time and varied by concentration. The 15% extract consistently slowed protein degradation, while the 25% and 35% concentrations produced sharply increased TVB-N, exceeding safety limits at later storage periods. This aligns with previous findings that phenolics act as antioxidants at moderate levels but can become pro-oxidants at higher levels (Apak et al., 2004), accelerating protein breakdown.

Similar patterns were reported by Nastiti et al. (2019). Mulberry fruit contains volatile compounds such as aldehydes, esters, ketones, benzene terpenes, and oxygenated terpenes (Sulistijowati, 2020). High TVB-N at the 25% concentration (e.g., 47.56 mg N/100 g) suggests accelerated degradation possibly due to protein instability, pH shifts, or altered colloidal properties (Campbell-Platt, 2009; Rozi, 2018). The 15% extract effectively lowered TVB-N at 36 h (17.93 mg N/100 g). Antioxidants in mulberry extract can bind free radicals and reduce alkaline compound formation (Nareswari, 2022). Fresh fish standards require TVB-N ≤20–30 mg N/100 g (BSN, 2013). Other natural preservatives, such as siam weed leaf and bay leaf extracts, demonstrated similar effects (Nurhamidah, 2020).

Protein result

The Kruskal-Wallis test revealed that extract concentration had a significant effect on protein level (p<0.001). The control and 15% groups had higher protein levels, while the 25% and 35% treatments were significantly lower. Protein levels decreased with storage, but the reduction was smallest at moderate extract concentrations. Higher concentrations (25%–35%) caused sharper declines due to protein denaturation from acidity and storage duration (Hikmah, 2020; Setiani et al., 2021). Extended storage leads to breakdown into simpler compounds and water-binding loss, making fish appear drier (Yuniati et al., 2024). Fresh carp typically contains 16–18% protein (Pratama et al., 2013). Carp immersed in a 15% extract for 30 min and stored for 24 h retained 16.4% of their protein content. Other natural preservatives, such as Pangium edule seeds and bay leaf extract, show similar protein-preserving potential (Ningrum et al., 2019).

Organoleptic test result

The results of the organoleptic test assessment of carp (*C. carpio*) treated with soaking red mulberry extract (*Morus alba*, L) at various concentrations are shown in the Table 3.

Table 3: Average results of organoleptic test assessment of goldfish (*Cyprinus carpio*) at various concentrations of red mulberry fruit extract (*Morus alba*, L)

No	The Extract Immersion	Eyes	Gills	Surface Mucus	Flesh	Odor	Texture	Overall Acceptance
1	0% Control, 12 h of Storage	7	7	7	8	7	7	7
2	0% Control, 24 h of Storage	6	6	6	7	5	7	6
3	0% Control, 36 h of Storage	3	5	5	6	3	6	4
4	15% Concentration, 12 h of Storage	8	9	8	8	8	8	8
5	15% Concentration, 24 h of Storage	6	7	6	7	7	7	7
6	15% Concentration, 36 h of Storage	6	6	5	6	5	5	5
7	25% Concentration, 12 h of Storage	6	6	6	6	7	7	6
8	25% Concentration, 24 h of Storage	5	6	6	5	5	5	5
9	25% Concentration, 36 h of Storage	4	6	5	4	5	4	4
10	35% Concentration, 12 h of Storage	8	8	8	7	7	7	7
11	35% Concentration, 24 h of Storage	5	6	6	5	5	5	5
12	35% Concentration, 36 h of Storage	5	4	5	4	4	4	4

From Table 3, the organoleptic scores were obtained from the assessment results of the fish's eyes, gills, surface mucus, flesh, odor, and texture, with a total of 6 assessing panellists. The sensory quality of the fish, evaluated through attributes such as eyes, gills, mucus, flesh, odor, and texture, was generally declined with longer storage.

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Fish treated with a moderate concentration of mulberry extract maintained higher organoleptic scores at shorter storage times, while higher extract concentrations and extended storage periods resulted in lower sensory values. Overall, the findings suggest that an optimal extract concentration can help preserve the sensory quality of fish, but prolonged storage diminishes these effects.

Although average organoleptic scores declined over time in all treatments, the 15% extract consistently preserved higher scores compared to the control. The organoleptic assessment showed that carp immersed in the 15% extract concentration maintained better quality than both the control and the higher concentrations. In contrast, immersion in the 25% and 35% concentrations for 24 and 36 h resulted in a noticeable decline in quality. This reduced efficacy at higher concentrations may be attributed to a decrease in the bioavailability of active compounds in highly concentrated plant extracts. At elevated levels, phenolic components may aggregate, precipitate, or form complexes that limit their interaction with bacterial cells, thereby diminishing antimicrobial activity despite the higher concentration (Marto, 2021). Excessive concentrations can also promote antagonistic interactions among phytochemicals, further lowering antibacterial effectiveness, whereas moderate concentrations preserve optimal solubility and activity (Sawalha, H, 2025). These mechanisms explain why the highest extract concentrations showed greater microbial growth compared to the lower concentration.

The red mulberry fruit extract has proven to increase the quality value of carp (*C. carpio*) to be stored at room temperature. A 15% extract concentration and 24 h immersion yielded the highest organoleptic score of 7, which meets the minimum threshold for fish deemed fit for consumption (BSN, 2013). This aligns with studies on other plant-based extracts used as natural preservatives. For instance, basil leaf extract (*Ocimum basilicum* L.) effectively preserved little tuna (*Euthynnus affinis*) (Marto, 2021). , and red pidada leaf extract (*Sonneratia caseolaris*), containing flavonoids, saponins, tannins, and phenols, showed significant antimicrobial and organoleptic benefits for milkfish (*Chanos chanos*) in assessments of the eyes, gills, slime, meat, and odor (Hikmah, 2020).

Conclusion

This study concludes that the extract of red mulberry fruit (*Morus alba* L.) contained active compounds, namely alkaloid, saponin, flavonoid, phenolic, and tannin which acted as antioxidant and antimicrobial substances. This phytochemical profile makes the extract a promising natural preservative for carp (*C. carpio*) maintaining the quality and edibility of the fish during room-temperature storage for up to 36 h. The most effective concentration was 15%. The result of the laboratory tests of pH test, TPC, organoleptic, TVB-N, and protein levels were all superior in the treated fish compared to the control. The stability of the active compounds at this concentration is likely responsible for its efficacy in preserving carp quality throughout the 36 h storage period.

It is suggested that further study would be conducted to determine the correlation between parameters and test concentrations below 15% to develop a more potent natural preservative.

Author contributions

I.S. conceptualized the study, curated the data, acquired funding, developed the methodology, supervised the project, and wrote the original draft; F.A.A. performed the formal analysis; Y.A.S. and I.S. conducted the investigation; S.A. administered the project and reviewed and edited the manuscript; I.S. and F.A.A. developed the software and visualization; Y.A.S. performed the validation; all authors read and approved the final manuscript.

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Conflict of interests

The authors declare that there is no conflict of interest.

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Ethical consideration

Not applicable.

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