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The Effect of Japanese Papaya Leaf Powder *(Cnidoscolus aconitifolius)* as a Natural Preservative of Block Fish During Cold Temperature Storage

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A B S T R A C T

Mackerel (Rastelliger Sp.) is a small pelagic fish commonly consumed as a source of animal protein. Due to its high susceptibility to spoilage, effective storage and the use of natural preservatives are essential to extend its shelf life. Japanese papaya leaf powder, which contains alkaloids, flavonoids, tannins, and saponins, has potential as a natural preservative due to its antimicrobial properties. This study aims to evaluate the optimal combination of Japanese papaya leaf powder concentration and storage duration on the quality of mackerel stored at low temperatures. A completely randomized factorial design was employed, with two factors: the concentration of Japanese papaya leaf powder (0%, 6%, 8%, and 10%) and storage duration (0, 2, 4, and 6 days). Data were analyzed using ANOVA at a 5% significance level, followed by Duncan's Multiple Range Test (DMRT) at the same significance level. The results indicated that the best treatment combination was 10% Japanese papaya leaf powder and 4 days of storage. This combination yielded a moisture content of 64.51%, a total microbial count of 6.6 cfu/g, a total volatile basic nitrogen (TVBN) level of 23.28 mg/100g, a pH of 5.34, a trimethylamine (TMA) level of 6.15 mg/100g, and a water holding capacity (WHC) of 53.11%. The organoleptic assessment showed that the mackerel maintained acceptable color and aroma, indicating effective preservation.

1. INTRODUCTION

1.1. Research Background

Fish is a food that has a high nutritional value content which includes protein, vitamins, and various minerals compared to other animal protein sources such as beef, mutton, chicken, and others. Mackerel (Rastelliger Sp.) is one type of small pelagic fish that lives on the surface of the water. The public widely consumes mackerel because it is a source of animal protein, tastes savory, is quite tasty, and is easy to process [1]. The high production of mackerel requires fishermen and traders to maintain the quality of the fish.

The problem in fisheries that still needs attention is how to handle fresh fish after being caught and consumed by consumers because the shelf life of fish is relatively fast 6 - 7 hours if not consumed immediately. The longer the storage, the more the total bacterial growth in fish increases due to microbial activity [2]. In general, bacteria that are often found in fresh fish are *Escherichia coli, Salmonella sp, and Vibrio cholerae* [3].

To overcome fish damage, a preservation process is carried out to extend the shelf life of fish. A commonly used preservation method is the use of low temperature or cooling using ice. Fish preservation treatment using low temperatures can affect the quality of fish quality both physically and biologically. Low-temperature conditions can slow down the growth of spoilage bacteria and biochemical processes that take place in the fish body [4]. However, the application of this method has many obstacles faced by fishermen because the storage time of fresh fish in a low-temperature environment is relatively short, increasing the risk of using hazardous chemicals such as borax and formalin [5]. The use of chemicals such as borax and formalin can have health impacts on consumers. The main danger posed by formalin when consumed by humans can cause cell damage or cell death which can trigger the growth of cancer cells [6].

To overcome the negative impact of using formalin, it is necessary to have additional treatments used in preserving fish products to extend the shelf life, one of which is the addition of natural preservatives derived from plants such as Japanese papaya leaves combined with preservation methods with cooling temperatures.

Japanese papaya *(Cnidoscolus aconitifolius)* is a plant that grows in tropical areas such as Asia and is usually used as a medicinal plant. Japanese papaya leaves can reduce cholesterol levels. Japanese papaya leaves contain several antimicrobial compounds such as alkaloids of 17.45 ± 0.65, tannins of 5.72 ± 0.00, saponins of 12.49 ± 0.021, and flavonoids 23.72 ± 0.02 which can potentially be used as one of the natural preservatives in fish products [7]. Plants containing tannins and flavonoids are antimicrobial compounds that can be used as natural preservatives in fish.

The mechanism of action of each compound is flavonoids inhibit nucleic acid synthesis, inhibit the function of the bacterial cytoplasmic membrane, and inhibit energy metabolism, alkaloids interfere with the constituent components of peptidoglycan in bacterial cells, saponins damage the cytoplasmic membrane in bacteria, while tannins have the activity of inhibiting or inactivating bacterial cells which can interfere with the rate of protein transport so that the bacterial cell wall is not perfectly formed. Low-temperature storage can inhibit microbial and enzyme activity and maintain the original properties of fresh fish [8].

Japanese papaya leaf powder has antimicrobial activity in inhibiting the growth of bacteria S. aureus and Pseudomonas aeruginosa with a 5 mm zone of inhibition each [9]. Basil leaf extract was able to inhibit the growth of Staphylococcus aureus bacteria with an inhibition zone diameter of 22.2-24.4 mm [10]. "Bangkok" papaya seed powder at concentrations of 6 grams, 8 grams, and 10 grams can inhibit bacterial growth. The greater the concentration of basil leaf extract, the higher the bacterial inhibition [11]. Based on this, this research was conducted to determine Japanese papaya leaf powder as a natural preservative for mackerel (Rastrelliger Sp.).

1.2. Literature Review

Mackerel (Rastelliger Sp.) is one type of small pelagic fish that lives on the surface of the water. Mackerel is widely consumed by the public because it is a source of animal protein, tastes savory, is quite tasty, and is easy to process [1]., Mackerel can last approximately 24 hours stored at cold temperatures. In this case, mackerel must be considered a good way of handling to maintain the quality of the fish because the shelf life of fish is relatively fast if not consumed immediately. cold storage still has limitations, namely the relatively short shelf life of meat. Fish storage at 15 ° C can last for 2 days. The longer the storage, the more the total bacterial growth in fish increases due to microbial activity. This is because fresh mackerel has a fairly high water content and protein content of 73.91% and 22.10%, making it a suitable medium for bacterial growth and accelerating the process of damage or decay in fish [12].

The decay process in fresh fish is caused by bacteria. Bacteria in fish come from the body of the fish that has been contaminated from the environment where it lives and even from the processing and storage environment. Bacteria commonly found in fish include Vibrio, Staphylococcus, Salmonella and Escherichia coli

One method that can be used to extend the shelf life of fresh fish is low temperature storage. Low temperature can slow down the growth of spoilage bacteria, besides that it can also inhibit biochemical processes that take place in the fish body [4]. In addition to the use of low temperature methods, there needs to be additional treatment in preserving fresh fish, namely by adding natural preservatives derived from plants. The utilization of plants as natural preservatives can prevent the use of chemicals in fish preservation.

Potential plants that are used and utilized as natural preservatives are Japanese papaya leaves *(Cnidoscolus aconitifolius)*. Japanese papaya leaves contain several antimicrobial compounds such as alkaloids, tannins, saponins, and flavonoids [7]. Plants that contain alkaloid, flavonoid, and saponin compounds are believed to prevent and inhibit bacterial growth.

Papaya "Bangkok" seed powder at concentrations of 6 grams, 8 grams, and 10 grams can inhibit bacterial growth for each storage time of 0 days, 2 days, 4 days, and 6 days. In addition, the average value of TVBN in the control and treatment of soaking basil leaf extract concentrations of 20%, 25%, and 30% was 19.87 mgN/100g, 18.33 mgN/100g, 16.62 mgN/100g and 14.18 mgN/100g [13]., respectively. The longer the time and the higher the concentration will produce lower TVBN values than other treatments.

Papaya seed powder at concentrations of 6 grams, 8 grams, and 10 grams has an average TPC value of $5.1x10^6$, $4.8x10^{6,}$ and $2.5x10^6$ CFU/gr which shows the amount of bacterial content below the quality standards of fresh fish (5×10^5) according to SNI No.01- 2729.1-2006 [11].

Japanese papaya leaf extract can inhibit Staphylococcus aureus with an average diameter of the inhibition zone at a concentration of 10% of 13.01 mm and positive control of 32.79 mm, respectively [14].

The mechanism for inhibiting bacterial growth from phytochemical compounds has different activities and functions. Flavonoid compounds are lipophilic which will damage the bacterial membrane. Flavonoids can inhibit energy metabolism by inhibiting the use of oxygen by bacteria. Energy is needed by bacteria for macromolecular biosynthesis, so if the metabolism is inhibited, the bacterial molecules cannot develop into complex molecules [15].

Alkaloids are a class of antibacterial compounds that can interfere with the constituent components of peptidoglycan in bacterial cells to inhibit bacterial growth. The mechanism of alkaloids in antimicrobial compounds is to inhibit the peptidoglycan component in bacterial cells which results in the shape of the cell wall layer being imperfect. The imperfect shape of the cell wall layer is due to the absence of peptidoglycan components so bacterial cells experience both physical and osmotic damage [16].

Tannins are phenol compounds that have the activity of inhibiting or inactivating bacterial cells which can interfere with the rate of protein transport so that the bacterial cell wall is formed imperfectly [17]. Tannins also have polypeptide targets that interfere with peptidoglycan synthesis so that cells are formed imperfectly.

The mechanism of action of saponins as antibacterial is to reduce surface tension resulting in increased permeability or cell

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leakage and resulting in intracellular compounds will come out [18].

1.3. Research Objective

This study aims to determine the effect of the concentration of Japanese papaya leaf powder and the length of storage at cold temperatures on mackerel.

2. MATERIALS AND METHODS

2.1. Materials and Tools

The materials used in this study were Japanese papaya leaves and mackerel fish. Materials used for analysis were Nutrient Agar (NA), amoxicillin, distilled water, pH 4 buffer solution, pH 7 buffer solution, Plate Count Agar (PCA), boric acid solution, saturated K2CO3 solution, HCI, 0.01N, 70% alcohol, and physiological saline solution (NaCI 0.85%).

Tools used for the preparation of Japanese papaya leaf powder include analytical balance, cabinet dryer, 60 mesh sieve, and blender. The tools used for analysis are analytical balance, tongs, mortar, glassware, filter paper, pH meter, plastic cup, autoclave, incubator, vortex, incase, stirrer, colony counter, petridish, ose, tweezers, test tube clamp, test tube rack, label paper, marker, pipette tip, and micropipette.

2.2. Design Experiment and Analysis

In this study using a completely randomized design (CRD) factorial pattern of 2 factors, factor I is the concentration of Japanese papaya leaf powder consisting of 4 levels (0%, 6%, 8%, and 10%). Factor II is the length of storage consisting of 4 levels (0 days, 2 days, 4 days, 6 days) each treatment is repeated twice. The data obtained were then analyzed using ANOVA (Analysis of Variance). If there is a significant difference between the treatments, it is continued with DMRT (Duncan's Multiple Range Test) further tests at a 5% level.

2.3. Implementation of Research

2.3.1. Raw Material Analysis

Fresh papaya leaf samples were weighed and washed to remove dirt and aeration. The samples were then dried in an oven at 70ºC for 1 day. After drying, it was crushed using a blender and filtered with a 60-mesh sieve to obtain powder. Analytical parameters include qualitative phytochemical tests and antibacterial activity using the hole/sound method.

2.3.2. Final Product Analysis

Fresh fish is washed using running water and then filleted. Japanese papaya leaf powder is weighed and then smeared on a fish meat fillet for 30 minutes. After smearing, it is drained, stored in a container, and placed at a temperature of 4-6°C. Analytical parameters include total microbes, water content, TVB, TMA, pH, and WHC.

3. RESULT AND DISCUSSION

3.1.1. Phytochemical Compounds

Table 1. shows that in Japanese papaya leaf powder powder is positive for alkaloid compounds, this is because when the solution is added to the powder, there is an orange precipitate. dragendorf reagent there is an orange precipitate. The mechanism of inhibition of bacterial growth of alkaloid antimicrobials works by inhibiting enzymes that play a role in the DNA replication process, which causes bacteria to be unable to replicate their DNA. enzymes that play a role in the DNA replication process which causes bacteria to be unable to divide it inhibits bacterial growth [19].

Table 1. Phytochemical content of Japanese papaya leaf powder powder

Types of phytochemical	Japanese Papaya Leaf Powder	
	Analyze	literature
Alkaloids	+	+
Flavonoids	+	+
Tannins	+	+
Saponins	+	+

The results of qualitative phytochemical analysis of Japanese papaya leaf powder are positive for flavonoid compounds, this is indicated by a change in color to yellow. The activity of flavonoid compounds against bacteria is carried out by damaging the wall consisting of lipids and amino acids. amino acids. The lipids and amino acids will react with the alcohol group on the flavonoid compound so that the cell wall will be damaged and the flavonoids enter the cell wall. On flavonoid compounds so that the cell wall will be damaged and flavonoids enter the nucleus of bacterial cells. into the bacterial cell nucleus [20].

The results of the analysis on Japanese papaya leaf powder showed that there are tannin compounds, this is indicated by a change in color to black greenish, or blue which indicates the

Formation of complex compounds between tannins and Fe^{3+}. and Fe^{3+}. The mechanism of tannins as antibacterial is by wrinkling the cell wall or cell membrane of bacteria, thus disrupting its cell permeability. As a result of the disruption of permeability, cells cannot carry out life activities so that their growth is inhibited, or even die [21].

The results of phytochemical analysis on Japanese papaya leaf powder showed that there were positive saponin compounds characterized by the formation of stable foam. The mechanism of saponin compounds as antibacterials is by reducing the surface tension of the bacterial cell wall which causes instability of the cell membrane thus inhibiting enzyme activity, especially enzymes that play a role in ion transport which is very important in the life of bacteria. If ion transport is inhibited, then bacterial growth will also be inhibited [22].

3.2. Antimicrobial Activity of Japanese Papaya Leaf Powder

Analysis of the antimicrobial activity of this study aims to determine the presence of antimicrobial activity in Japanese papaya leaf powder by using the method of pitting method. Antimicrobial activity is indicated by the formation of a clear zone around the hole / well.

Table 2 shows the results of antimicrobial activity analysis on Japanese papaya leaf powder. The average diameter of the inhibition zone for each treatment of Japanese papaya leaf powder is 5.25 mm while for the control treatment (+) amoxicillin was 9.65 mm. The range of inhibition zone values less than 5 mm is classified as weak, the range of inhibition zones between 5-10 mm is classified as strong, and 11-20 mm is classified as very strong [23].

Table 2. Inhibition Zone Diameter Results of Japanese Papaya Leaf Powder Against Mackerel Contamination

Japanese Papaya Leaf Powder Concentration (%)	Inhibition Zone Diameter (mm)	Literature
Amoxicillin (control +)	9.65	32.79
Leaf Powder 10%	5.25	13.01

Based on Table 2, Japanese papaya leaf powder in inhibiting antibacterial activity characterized by a clear zone around the area is classified as strong.

3.3. Total Microbes

The average total microbes of mackerel in Table 3. ranged from 5.58 - 5.59 cfu/g. The treatment of 8% Japanese papaya leaf powder mixture concentration at 0 days storage resulted in the lowest total microbes of 5.58 cfu/g, while the treatment of 0% Japanese papaya leaf powder mixture concentration at 6 days storage resulted in the highest total microbes of 5.99 cfu/g.

Table 3. Phytochemical content of mackerel

Japanese Papaya Leaf Powder Concentration (%)	Storing Time (Days)	Total Microbes	Notation
0	0	5.60 ± 0.13	B
	2	5.78 ± 0.14	E
	4	5.93 ± 0.00	E
	6	5.99 ± 0.01	F
6	0	5.61 ± 0.10	B
	2	5.77 ± 0.05	D
	4	5.75 ± 0.03	D
	6	5.80 ± 0.01	E
8	0	5.58 ± 0.02	A
	2	5.65 ± 0.01	C
	4	5.74 ± 0.05	D
	6	5.83 ± 0.02	E
10	0	5.63 ± 0.02	C
	2	5.63 ± 0.01	C
	4	5.68 ± 0.05	C
	6	5.79 ± 0.07	E

Notes: Mean values accompanied by different letters mean significantly different at $p \leq 0.05$

It has been demonstrated that higher concentrations of Japanese papaya leaf powder result in lower total microbial counts in mackerel. However, extended storage leads to an increase in microbial counts. This indicates that the powder effectively inhibits microbial growth in mackerel during low-temperature storage. The effectiveness of these powders is attributed to their antimicrobial compounds, such as flavonoids, tannins, and alkaloids. Flavonoids, in particular, are antibacterial agents that disrupt the function of bacterial cytoplasmic membranes and inhibit nucleic acid synthesis. The inhibition of nucleic acid synthesis is done by inhibiting transcription and replication of microorganisms. This is also reinforced by the statement [25], that alkaloids can inhibit the synthesis of nucleic acids and enzymes that play a role in the DNA replication process, thus inhibiting bacterial growth. inhibit bacterial growth.

During cold storage, the total microbial count of mackerel is, however, the higher the concentration of Japanese papaya concentration of Japanese papaya leaf powder, the lower the total microbes in mackerel fillets. Total microbes in mackerel increased during storage time due to the increase in water content during storage which is used by microbes as a growth medium. This is following Ref. [26], which states that the increase in total microbes during storage is related to the water content which also increases during storage.

3.4. Water Content

The average water content of mackerel ranges from 73.58 to 79.45% (Table 4). The lowest moisture content of 73.58% was the treatment of 10 g Japanese papaya leaf powder mixture concentration at 0 days storage, while the treatment of 0 g Japanese papaya leaf powder mixture concentration at 6 days storage produced the highest moisture content of 79.45%.

Table 4. The water content of mackerel

Japanese Papaya Leaf Powder Concentration (%)	Storing Time (Days)	Water Content	Notation
0	0	76.00 ± 0.28	d
	2	77.79 ± 0.17	g
	4	79.14 ± 0.50	g
	6	79.45 ± 0.64	h
6	0	75.47 ± 0.32	c
	2	78.06 ± 0.47	g
	4	78.29 ± 0.89	g
	6	78.55 ± 0.95	g
8	0	74.56 ± 0.84	b
	2	75.38 ± 1.01	c
	4	77.62 ± 0.12	g
	6	78.52 ± 1.00	g
10	0	73.58 ± 1.00	a
	2	76.52 ± 0.96	e
	4	76.91 ± 0.64	f
	6	78.28 ± 0.89	g

Notes: Mean values accompanied by different letters mean significantly different at $p \leq 0.05$

Studies have shown that increasing the concentration of Japanese papaya leaf powder reduces the water content in mackerel. However, prolonged storage leads to an increase in water content. This initial decrease in water content is attributed to the antimicrobial properties of the Japanese papaya leaf powder, which inhibit bacterial protein degradation and thus prevent the release of bound water in the fish. This is supported by Ref. [27], that the addition of antimicrobial ingredients can inhibit the breakdown of proteins by bacteria which results in the breakdown of protein structures which have an impact on the release of bound water. These antimicrobial compounds will change the components that make up bacterial cells. which will change the constituent components of the bacterial cell itself so that it is inhibited from growing.

In addition, the increase in water content in the sample after treatment was due to the effect of the addition of natural preservatives that can withstand the release of bound water by inhibiting bacterial growth. the release of bound water by inhibiting bacterial growth. The longer the storage there is an increase in moisture content in mackerel fillets. This is caused by the growth of microbes in the fish, the microbes will decompose nutrients in fish to produce microbial metabolic products in the

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form of H2O or water vapor. The water content in fish is interrelated with an increase in the number of bacteria in the fish [28]. In fish is interrelated with an increase in the number of bacteria. The addition of addition of antimicrobial ingredients can inhibit the rate of protein breakdown by bacteria, which results in the breakdown of the protein structure. resulting in the breakdown of the protein structure which has an impact on the release of bound water. bound. In addition, the increase in moisture content during storage is caused by several factors such as the initial moisture content of the ingredients, the high humidity of the surrounding environment, and microbial growth in mackerel. microbial growth in mackerel.

3.5. Total Volatile Base Nitrogen

The average TVBN value of mackerel is in Table 5. Ranges from 19.40 -28.75 mg/100g. Based on the TVBN value obtained, mackerel with a storage period of 0 days to 6 days is still considered suitable for consumption. The lowest TVBN value of 19.40 mg/100g was the treatment of 10% Japanese papaya leaf powder concentration at 0 days storage, while the treatment of 0% Japanese papaya leaf powder concentration at 6 days storage produced the highest TVBN value of 28.75 mg/100g. The TVBN value between 20-30 mg/100g includes criteria that can be consumed and if more than 30 mg/100g, it includes criteria that cannot be consumed [29].

Table 5. Total Volatile Base Nitrogen of mackerel

Japanese Papaya Leaf Powder Concentration (%)	Storing Time (Days)	TVBN	Notation
0	0	19.40 ± 0.46	A
	2	23.78 ± 0.54	De
	4	25.27 ± 0.09	F
	6	28.75 ± 1.07	G
6	0	20.05 ± 0.17	Ab
	2	22.50 ± 0.28	C
	4	24.36 ± 0.05	E
	6	25.46 ± 0.19	F
8	0	20.25 ± 0.07	B
	2	22.21 ± 0.23	C
	4	24.13 ± 0.01	E
	6	25.25 ± 0.07	F
10	0	20.05 ± 0.05	Ab
	2	21.96 ± 0.06	C
	4	23.28 ± 0.16	D
	6	25.27 ± 0.03	F

Notes: Mean values accompanied by different letters mean significantly different at p ≤ 0,05

The higher the concentration of Japanese papaya leaf powder can reduce the TVBN value, but the longer the storage of mackerel, the TVBN value will increase. The decrease in TVBN value in mackerel is due to antimicrobial compounds in the form of flavonoids, tannins, and alkaloids in Japanese papaya leaves which can inhibit the activity of proteolytic enzymes produced by bacteria in breaking down proteins into nitrogen compounds in fish meat. The higher the activity of proteolytic enzymes, the TVB value will also increase. Phytochemical compounds can reduce enzyme and biochemical activity by forming phenolic protein complexes with enzymes [30]. The low TVB-N value is due to the content of water-soluble flavonoids that easily seep into fish meat tissue and are able to inhibit enzyme activity and biochemical activity in fish meat [31], while tannins also can activate enzymes so that they can inhibit enzyme activity in fish meat tissue.

The longer the storage time of mackerel, the higher the TVBN value. increases. This is due to microbial activity, the length of storage in fish results in the growth of microbes that break down proteins to produce several volatile bases such as NH3, indole, H_2S, and TMA. produces a number of volatile bases such as NH3, indole, H_2S, and TMA. Ref. [32] stated that the TVBN value increases with increasing storage time. as storage time increases. This is because TVBN is a compound resulting from protein degradation due to enzyme activity and spoilage bacteria. An increase in TVBN concentration is associated with microbial growth and can be used as an indicator of fish spoilage. can be used as an indicator of fish damage. The large number of microbes in fish makes the process of protein degradation into nitrogenous base compounds faster, so the concentration of TVBN also increases. so that the concentration of TVBN also increases.

3.6. Trimetylamine(TMA)

The average TMA value of mackerel in Table 6. ranged from 5.07 - 9.12 mg/100g. The lowest TMA value of 5.07 mg/100g was the treatment of 0% Japanese papaya leaf powder concentration at 0 days storage, while the treatment of 0% Japanese papaya leaf powder concentration at 6 days storage produced the highest TMA value of 9.12 mg/100g.

Table 6. TMA Value of mackerel

Japanese Papaya Leaf Powder Concentration (%)	Storing Time (Days)	TMA	Notation
0	0	5.20 ± 0.01	a
	2	6.23 ± 0.12	c
	4	7.60 ± 0.21	f
	6	9.01 ± 0.15	h
6	0	5.09 ± 0.02	a
	2	6.19 ± 0.17	c
	4	6.68 ± 0.02	d
	6	8.61 ± 0.02	g
8	0	5.20 ± 0.04	a
	2	5.88 ± 0.00	b
	4	6.30 ± 0.02	c
	6	7.09 ± 0.01	e
10	0	5.17 ± 0.04	a
	2	5.80 ± 0.02	b
	4	6.15 ± 0.03	c
	6	6.84 ± 0.07	d

Notes: Mean values accompanied by different letters mean significantly different at p ≤ 0,05

The higher the concentration of Japanese papaya leaf powder can reduce the TMA value, but the longer the storage of mackerel the TMA value increases. This indicates that during storage there are changes in fish quality that lead to spoilage which is characterized by an increase in TMA value, This can be seen in Table 6. The longer the storage and the higher the concentration of Japanese papaya leaf powder, the lower the TMA value, this is due to the phytochemical compounds contained in Japanese papaya leaf powder which are antibacterial. This phytochemical compound is able to inhibit the activity of enzymes derived from bacteria in fish, basically bacteria have proteolytic enzymes derived from fish meat. Proteolytic enzymes derived from fish meat that will hydrolyze proteins into amino acids and break down back into other nitrogen compounds that produce volatile bases. This is in accordance with Ref. [33], TMA is part of TVB which causes the TMA content to always be lower than TVB, TMA is formed from the decomposition of protein by bacteria. TMA is formed from the decomposition of proteins by the activity of bacteria and enzymes, as a result of the breakdown of proteins into bases with a pH of 7.1-7.2. The breakdown results are volatile and give rise to foul-smelling compounds such as NH_3, N_2S, mercaptan, cresol, indole, and scotol. such as NH_3, N_2S, mercaptan, cresol, indole and scotol.

Increased TMA values are caused by bacterial activity activity in dead fish material that reduces TMAO to TMA. The decomposition of TMAO into TMA after the fish dies will produce ammonia which affects the aroma and flavor, causing a rotten smell. TMA will increase faster the longer the storage time. This is because in fish meat there is trimethylamine and ammonia. According to Ref. [34], the decomposition of protein in fish will produce trimethylamine and ammonia compounds. If the freshness of the fish decreases, the volatile nitrogen content will increase.

3.7. pH

The average pH value of mackerel in Table ranges from 5.26 to 7.19. The lowest pH value of 5.27 was the treatment of Japanese papaya leaf powder concentration of 10% at 2 days of storage. powder concentration at 2 days of storage, while the treatment of concentration of concentration treatment of 0% Japanese papaya leaf powder at 6 days of storage resulted in the highest pH value of 7.19. the highest pH value of 7.19.

Table 7. pH Value of mackerel

Japanese Papaya Leaf Powder Concentration (%)	Storing Time (Days)	pH	Notation
0	0	6.08 ± 0.26	d
	2	6.45 ± 0.48	e
	4	6.80 ± 0.00	f
	6	7.19 ± 0.04	g
6	0	5.89 ± 0.28	d
	2	5.62 ± 0.22	c
	4	5.71 ± 0.16	c
	6	5.80 ± 0.26	c
8	0	6.10 ± 0.41	d
	2	5.26 ± 0.16	a
	4	5.39 ± 0.04	b
	6	5.88 ± 0.48	d
10	0	6.08 ± 0.10	d
	2	5.27 ± 0.09	a
	4	5.34 ± 0.24	b
	6	5.54 ± 0.04	c

Notes: Mean values accompanied by different letters mean significantly different at $p \leq 0,05$

Table 7 shows that the higher the concentration of Japanese papaya leaf powder, the lower the pH. papaya leaf powder can reduce the pH, while the longer the storage of mackerel pH increases. This is due to the presence of phytochemical compounds that can inhibit bacterial growth. An increase in pH indicates microbial activity that breaks down protein and fat to produce alkaline compounds such as ammonia. produce alkaline compounds such as ammonia. This is also supported by Ref. [35], The increase in pH value during storage is due to the accumulation of basic compounds, such as ammonia derived from microbial activity. In addition, the decrease in pH in soaking is due to the formation of lactic acid as a result of the reaction of glycogen breakdown by enzymes contained in fish meat. According to Ref.[36], the decrease in pH value is due to the breakdown of glycogen in meat into lactic acids. into lactic acids, the longer the storage, the higher the lactic acid formed from the decomposition formed from the decomposition is getting higher, and there will be an accumulation of lactic acid. lactic acid.

The pH of mackerel is also influenced by the length of storage, the longer the storage, the more the pH value increases. Ref. [37] added that the increase in pH in fish meat during storage indicates the presence of microbial activity contained in fish meat tissue so that ammonia compounds are formed. alkaline ammonia compounds. The longer the storage, the increase in pH value in the early post-rigor phase, and continues to increase in the late post-rigor phase.

3.8. Water Holding Capacity

The average WHC of mackerel in Table 8. ranges from 38.67 - 56.61%. Treatment of Japanese papaya leaf powder concentration of 0% at 6 days storage produced the lowest WHC of 38.67%, while the treatment of concentration concentration treatment of 8% Japanese papaya leaf powder at 0 days storage produced the highest WHC of 56.61%.

Table 8. Water Holding Capacity of mackerel

Japanese Papaya Leaf Powder Concentration (%)	Storing Time (Days)	WHC	Notation
0	0	50.90 ± 0.76	g
	2	46.50 ± 0.99	e
	4	45.71 ± 0.74	d
	6	38.67 ± 0.24	a
6	0	43.09 ± 0.24	i
	2	45.72 ± 0.86	d
	4	43.18 ± 0.64	b
	6	39.96 ± 0.67	a
8	0	56.61 ± 0.31	j
	2	48.09 ± 0.02	f
	4	44.68 ± 0.60	c
	6	44.99 ± 0.70	d
10	0	55.48 ± 0.55	i
	2	51.51 ± 0.49	h
	4	52.42 ± 1.02	i
	6	47.79 ± 0.82	f

Notes: Mean values accompanied by different letters mean significantly different at $p \leq 0.05$

Table 8 shows that a higher concentration of Japanese papaya leaf powder can increase WHC. but the longer the storage of mackerel WHC decreases. This is due to microbial activity. the addition of Japanese papaya leaf powder can slow down the decrease in the water-holding capacity of mackerel meat. This is because Japanese papaya leaf powder contains antimicrobial compounds that cause microbial growth to be inhibited so that protein damage does not occur. which results in decreased water holding capacity. Tannin and flavonoids play a role in inhibiting microbial growth by interfering with the membrane formation process to inhibit the process of membrane formation so that it can inhibit growth and even kill bacteria. Tannin compounds that bind to proteins will produce insoluble compounds and make the proteins in the meat difficult to break down insoluble compounds and make the protein in the meat difficult to break down [38]. because proteins that are difficult to break down result in the strength of water holding power being maintained. maintained.

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The longer the storage. the lower the WHC in mackerel. this is due to the presence of proteolytic bacteria in mackerel during storage which causes bacterial activity to hydrolyze proteins that produce other volatile base compounds. so that the higher the total bacteria. the lower the ability to bind water. According to Ref. [39]. the hydrolysis of meat protein by proteolytic enzymes causes the volume of muscle fibers to expand so that the binding power of water decreases. the volume of muscle fibers expands so that the water-holding capacity decreases. In addition. during storage. protein denaturation occurs which causes a reduction in the hydrophilic hydrophilic groups so that the ability to bind water is also reduced. According to [40]. during storage, there will be denaturation of collagen from the protein that cross-links between meat fibers. and it is stated that the main component that functions to bind water is protein. The main component that functions to bind water is protein as time increases time. there are changes in the protein structure in fish meat that can weaken the ability of the meat to bind to water. weaken the ability of meat to bind water.

4. CONCLUSION

The treatment of the addition of Japanese papaya leaf powder concentration and storage duration of mackerel had a significant effect on total microbes. water content. TVBN. trimethylamine. ph. and WHC. Mackerel with Japanese papaya leaf powder concentration treatment is still suitable for consumption until the 4th day of storage with total microbes 5.68 cfu/g. 76.91% moisture content. TVBN of 23.28 mg/100g. Trimethylamine of 6.15 mg/100g, pH of 5.34. and WHC of 52.42%.

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