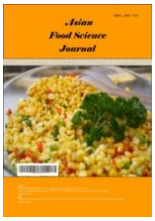

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Effectiveness of the Ruku-ruku Leaf Solution (*Ocimum sanctum*) as a Natural Preservative in Indian Mackerel (*Rastrelliger* sp.) during Low-temperature Storage

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Authors' contributions

This work was carried out in collaboration among all authors. Author MHR designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Author IR correcting the deficiencies in the first draft and managed the analyses of the study. Authors YA and RIP correcting the deficiencies in the first draft and managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

This research was conducted to determine the shelf life of Indian mackerel by giving ruku-ruku leaf solution at different concentrations on phytochemical test, amount of bacteria, degree of acidity (pH), weight loss, and water content in Indian mackerel during low-temperature storage. The study was conducted at the Laboratory of Fisheries Product Processing, University of Padjadjaran, Jatinangor. The research method used is an experimental method with 4 treatments. Ruku-ruku leaf solution treatments concentration were 0%, 10%, 30%, and 50%, soaking time 30 minutes, then stored at low-temperature (5-10ºC). Observations were made on days 1, 3, 6, and 7 for Indian mackerel concentration of 0% or control (without soaking of ruku-ruku leaf solution) while treatment with ruku-ruku leaf solution 10%, 30%, and 50% were carried out at days 1, 3, 6, 7, 8, 9, 10, 11,

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12, and 13. The parameters observed included phytochemical test, amount of bacteria, degree of acidity (pH), weight loss, and water content. The results of research showed that the use of a ruku-ruku leaf solution with concentration 30% on Indian mackerel during low-temperature storage has the longest shelf life that is until the 13th day with amount of bacteria 6,90 × 10^7 cfu/g, degree of acidity (pH) at 6,95, weight loss at 9,52% and water content at 65,32%.

Keywords: concentration; Indian mackerel; low-temperature; ruku-ruku leaf solution.

1. INTRODUCTION

Fish is a source of protein, containing fatty acids not containing long-term fat, containing vitamins, as well as macro and micro minerals which contain meaning for human health [1]. Indian mackerel is one type of seawater fish that is of interest to Indonesian people as consumption fish because besides having a good taste of Indian mackerel is also classified as a very economical fish so that it can be reached by people from various walks of life [2]. Fish is a functional food that is easily damaged by biological enzymes or spoilage microbiology, so it requires special handling to maintain its quality [3].

Efforts that can be done to maintain the freshness of fish for a long time is by handling low-temperature (5-10^{o}C). The use of low-temperature in fishery products can inhibit enzyme activity and bacterial growth, so that deterioration in fish quality will run much slower and fish will remain fresh for a long time [4]. Another effort that can maintain the quality of fresh Indian mackerel is the combination of cooling and preserving. Preservatives are compounds that can inhibit and stop fermentation, acidification, or other forms of damage, or materials that can provide food protection from spoilage [5].

According to Parnanto et al. [6] natural ingredients have the potential to preserve fish, because natural ingredients have microbial inhibiting activity caused by certain components in them. Ditjen POM [7] states that ruku-ruku leaves contain 4,6% tannins, 2% essential oils, alkaloids, flavonoids, steroids/triterpenes, saponins, and glycosides. The chemical content of ruku-ruku plants has antioxidant, antimicrobial, antimutagen and anti-allergic properties [8]. The ruku-ruku act as an anti-bacterial especially in *Escherichia coli, Streptococcus mutans* and *Staphylococcus aureus,* even ruku-ruku leaf extract was reported to show strong antifungal activity against *Aspergillus* sp. [9]. The water extract of the ruku-ruku plant showed inhibition of the growth of *Klebsiella* sp., *Escherichia coli*, *Proteus* sp. and *Staphylococcus aureus,* even alcoholic extracts of ruku-ruku can inhibit the growth of *Vibrio cholera* [10].

This research aims to determine the shelf life of Indian mackerel by giving a solution of ruku-ruku leaves at different concentrations of amount of bacteria, degree of acidity (pH), weight loss, water content and phytochemical test in Indian mackerel during low-temperature storage.

2. MATERIALS AND METHODS

2.1 Time and Place of Research

Research was carried out from May to July 2019 at the Laboratory of Fisheries Product Processing, Faculty of Fisheries and Marine Sciences, then testing water content at the Animal Nutrition & Ruminant Chemical Nutrition Laboratory, Faculty of Animal Husbandry, and phytochemical testing at the Laboratory of Chemical Applications and Services, PPBS, University of Padjadjaran, Indonesia (107º45' 8,5" – 107º48' 11,0" ES and 6º53' 43,3" – 6º57' 41,0" SL).

2.2 Tools and Materials

The tools used in this research are coolbox for the transportation and storage of ice and Indian mackerel, digital scales for weighing ruku-ruku leaves, scissors, blenders, filters, glass jar, dipper scale, petridish for bacteria incubation containers, porcelain cups and mortar, plastic pipette, test tube, hotplate, magnetic stirrer, autoclave, erlenmeyer, analytic scales, desiccator, incubator, bunsen, spatula, colony counter, pH meter, beaker glass, cling wrap, aluminum foil, tissue towel, perforated plastic, refrigerator (5-10^{o}C), styrofoam plates, drainers, ovens, stationery, labels, cameras, basins, porcelain cups and mortars. While the materials used are Indian mackerel, ruku-ruku leaves, curai ice, aquadest, hand sanitizer, standard buffer pH 4 and 7, physiological NaCl solution, agar nutrient, and alcohol.

2.3 Observation Parameters

Observations were made for 13 days after the soaking process was carried out. Observation of Indian mackerel for treatment with soaking of ruku-ruku leaf solution was carried out on storage days 1, 3, 6, 7, 8, 9, 10, 11, 12, and 13 whereas for Indian mackerel treatment without soaking of ruku-ruku leaf solution conducted on storage days 1, 3, 6, and 7. The parameters observed in this research were phytochemical test, amount of bacteria, degree of acidity (pH), weight loss, and water content.

2.3.1 Phytochemical test

Phytochemicals are science that describes the chemical aspects of a plant, the study of phytochemicals include a description that encompasses a wide variety of organic compounds that are formed and stored by the organism, the chemical structure, biosynthesis, changes and metabolism, distribution natural and biological function, isolation and comparison of the composition of compound chemistry of various types of plants [11]. The phytochemical testing of the ruku-ruku leaves is carried out by various methods listed in Table 1.

2.3.2 Amount of bacteria

The following formula Fardiaz [13] is used to calculation for amount of bacteria colonies:

Colonies per ml= (Number of colonies per cup × (1/Dilution factor))

2.3.3 Acidity (pH)

According to Widiani [14], the pH value measurement procedure is as follows:

1) Samples of 5 grams fish meat is crushed until smooth.
2) Samples are put into a test tube containing 45 mL of aquadest.
3) Samples are shaken until homogeneous, then measured with a pH meter that has been calibrated to a standard buffer of pH 4 and pH 7.

2.3.4 Weight loss

The formula of Afrianto and Liviawaty [15] was used to calculate the weight loss as follows:

Shrinkage of fish weight = (initial weight-final weight / initial weight) × 100%

2.3.5 Water content

Calculation of water content can be do by using AOAC [16] formula as follows:

Water Content = (B1-B2 / B) ×100%

Information: B = Sample weight (gram), B1 = Weight (sample + cup) before drying, B2 = Weight (sample + cup) after drying

2.4 Data Analysis

Observational data of phytochemical test, amount of bacteria (TPC), degree of acidity (pH), weight loss and water content were analyzed descriptively and presented in tables and curves based on the results of phytochemical test, amount of bacteria, pH, weight loss and water content Indian mackerel during low-temperature storage.

3. RESULTS AND DISCUSSION

3.1 Phytochemical Test

The results of phytochemical testing of ruku-ruku leaves are presented in Table 2. Phytochemical testing is one of the important steps in efforts to uncover the potential efforts of plant resources. The results of phytochemical analysis can provide clues about the presence of chemical components (compounds) of the type of alkaloid, flavonoid, phenolic, steroid, and triterpenoid species in plants [17].

Tannins in the leaves of ruku-ruku has the ability to bacteriostatic and bakteriocidal. Tannins work by holding a hydrophobic complex with proteins, inactivating adhesin, enzymes and cell wall transport proteins that interfere with the growth of microorganisms [18].

Flavonoids are also plant defense compounds that inhibit the appetite of insects (*antifeedants*) and are toxic [19]. In addition, Cushnie and Lamb [20] revealed that as an antibacterial, flavonoids do not kill bacterial cells but induce the formation of bacterial aggregates thereby reducing the amount of colonies. The mechanism is to inhibit nucleic acid synthesis, inhibit cytoplasmic membrane function and inhibit energy metabolism.

Saponins are triterpenoid glycosides and sterols. Saponins are compounds that taste bitter, foam in water and are soluble in water and alcohol but are not soluble in ether [21].

Table 1. Phytochemical test method for Ruku-ruku leaves

No.	Secondary Metabolites	Method Test
1.	Phenolic	$FeCl_3$ reagent (5%)
2.	Tannins	$FeCl_3$ reagent (1%)
3.	Flavonoids	a. Concentrated HCl reagent + Mg b. H_2SO_4 reagent (2N) c. NaOH reagent (10%)
4.	Saponins	HCl reagent (2N)
5.	Triterpenoids Steroids	Concentrated H_2SO_4 reagent + CH_3COOH anhydrous
6.	Alkaloids	a. Dragendorff reagent b. Wagner reagent

Source: [12]

Table 2. Phytochemical test results of Ruku-ruku leaves

No.	Secondary Metabolites	Test Results
1.	Phenolic	-
2.	Tannins	+
3.	Flavonoids	+
4.	Saponins	+
5.	Triterpenoids	+
6.	Steroids	-
7.	Alkaloids	+

Information: + = Available, - = Not Available

Table 3. Amount of bacteria in Indian mackerel during storage

Days to-	Concentration			
	0%	10%	30%	50%
1	$2{,}62 \times 10^3$	$2{,}90 \times 10^3$	$2{,}68 \times 10^3$	$2{,}48 \times 10^3$
3	$2{,}24 \times 10^4$	$3{,}90 \times 10^3$	$3{,}14 \times 10^3$	$3{,}57 \times 10^3$
6	$2{,}65 \times 10^6$	$2{,}81 \times 10^5$	$2{,}96 \times 10^4$	$2{,}90 \times 10^4$
7	$1{,}61 \times 10^7$	$2{,}88 \times 10^6$	$2{,}69 \times 10^5$	$2{,}93 \times 10^5$
8	-	$9{,}35 \times 10^6$	$5{,}65 \times 10^5$	$5{,}50 \times 10^5$
9	-	$1{,}70 \times 10^7$	$1{,}20 \times 10^6$	$1{,}55 \times 10^6$
10	-	-	$1{,}50 \times 10^6$	$1{,}94 \times 10^6$
11	-	-	$1{,}76 \times 10^6$	$2{,}40 \times 10^6$
12	-	-	$2{,}09 \times 10^6$	$5{,}00 \times 10^6$
13	-	-	$6{,}90 \times 10^7$	$5{,}10 \times 10^7$

Information: (-) = Not Observed

Saponins can be used as poisons and antimicrobials (fungi, bacteria, and viruses). Saponin as an antimicrobial is due to its ability to cause leakage of certain proteins and enzymes from cells [22].

Triterpenoids are colorless in crystal form, high melting point and optically active. These compounds have a cyclic structure and are mostly alcohol, aldehyde, or carboxylic acids. The mechanism of its antibacterial is reacted with Porin (protein transmembrane) on the outer membrane of the bacterial cell wall, forming strong bond polymers resulting in the destruction of Porin [23]. Triterpenoids are also lipophilic which can damage bacterial cell membranes, but these compounds are reported to only be able to inhibit bacteria as much as 30% of total microorganisms [23].

Alkaloids have the ability to be antibacterial, the mechanism of which is to interfere with the constituent components of peptidoglycan in bacterial cells, so that the cell wall layers are not formed intact and cause cell death [24]. In addition, alcaloid is also able to interact with bacterial DNA, causing DNA damage and bacterial lysis [23].

The mechanism of action of antibacterial agents in inhibiting growth is influenced by several factors including the concentration of antibacterial substances, storage time, environmental temperature, environmental pH , and bacterial properties including age, type and condition of bacteria [13].

3.2 Amount of Bacteria

The results of observing amount of Indian mackerel bacteria during low-temperature storage are presented in Table 3. Calculation on amount of bacteria is used to estimate the shelf life of foodstuffs and as an indicator of food sanitation. Microbiology is an important factor in food products, one of the microbiological characteristics of a food can be seen from amount of bacteria. Amount of bacteria can be an indicator of the freshness of Indian mackerel and determine its shelf life.

Based on observations of day 1 seen in all treatments given ruku-ruku leaf solution, amount of bacteria was not too large. This is because bacteria are still in the *lag* phase or adaptation which is a phase of bacteria that is still adjusting to the new environment, so that the cell has not yet divided [25]. Bacteria are also still adjusting to temperature differences and the presence of antimicrobials in Indian mackerel from the ruku-ruku leaf solution. The greater the difference between the temperature in the fish habitat with the storage temperature used, the bacterial growth is increasingly inhibited [26].

The results of observations on day 3, amount of Indian mackerel bacteria by soaking the ruku-ruku leaf solution at a concentration of 10%, 30%, and 50% had amount of bacteria respectively 3,90 × 10^3 cfu/g, 3,14×10^3 cfu/g, and 3,57×10^3 cfu/g, amount of bacteria is less than that of Indian mackerel without soaking the ruku-ruku leaf solution (control), which is equal to 2,24 × 10^4 cfu/g, the same results in research by Dewi et al. [27] on the 3rd day, amount of bacteria in control Indian mackerel during cold storage (4ºC) also had a bacterial count of 1,03 ×10^4 cfu/g. Increasing amount of spoilage bacteria in control Indian mackerel is more significant, this is due to the absence of antimicrobials such as tannins, flavonoids, saponins, triterpenoids, and alkaloids (Table 2), which work to inhibit bacterial growth.

Amount of Indian mackerel bacteria increases with the duration of storage. An increase in amount of bacteria from the third day of storage until the end of the storage shows that the bacteria have been able to adapt to the medium so that they can grow and multiply. After the *lag* or adaptation phase, bacteria will enter the logarithmic or exponential phase, in this phase, the cell has been dividing at a constant rate, the mass has doubled at the same rate, balanced metabolic activity, and balanced growth. Balanced growth is characterized by regular population increase [28].

The growth of spoilage bacteria in the growth phase is influenced by the antimicrobial content contained in the ruku-ruku leaves, this is seen in the amount of spoilage bacteria starting from the 6th day of storage in control Indian mackerel is greater, reaching 2,65 × 10^6 cfu/g with acceptance limit until the 7th day, compared to Indian mackerel with soaking ruku-ruku leaf solution concentration of 10%, 30%, and 50%, on the 6th day amount of bacteria each only reached 2,81×10^5 cfu/g, 2,96×10^4 cfu/g, and 2,90×10^4 cfu/g, and with consecutive acceptance limits up to the 9th, 13th, and 13th days. The limit on receiving amount of bacteria in Indian mackerel refers to Connel [29] that amount of bacteria in food that can be consumed is 10^6 cfu/g, then if amount of bacteria reaches 10^7 cfu/g then Indian mackerel is not suitable for human consumption and the previous day is the last limit of the shelf life.

The difference in amount of days at the 10% concentration limit can be due to too few antimicrobial compounds so that they are less effective in inhibiting bacterial growth. Meanwhile, concentrations of 30% and 50% do have the same acceptance limit for amount of bacteria, but the most effective concentration is a concentration of 30%, because its efficiency and function are more optimal in inhibiting microbial growth. As for the concentration of 50% to be ineffective because the addition of the concentration of the solution does not always provide a stronger bacterial growth-inhibiting effect.

Ganiswarna [30] states that an increase in the concentration of a material will be followed by an increase in the inhibition of bacterial growth, but at the maximum concentration there will be a decrease in the inhibition of bacterial growth. Addition of concentration does not provide a longer shelf life due to the content of nitrogen and protein compounds contained in

extracts at a certain amount utilized by bacteria and spur growth [31]. Bacteria can fix nitrogen and collect it in the form of compounds in their cells [32]. This shows that increasing the concentration of the ruku-ruku leaf solution does not always provide a better inhibitory effect on bacterial growth.

3.3 Acidity (pH)

The results of observing the acidity (pH) of Indian mackerel during low-temperature storage are presented in Fig. 1. Measurements of pH value of Indian mackerel do (duplo) by using a pH meter. Measurements of pH are carried out to determine chemical changes during storage. The degree of acidity is important to be used as an indicator in determining the level of freshness of fish that affects the storage time.

After the fish die, degree of acidity (pH) tends to decrease [33]. The degree of acidity after the fish dies ranges from 6-6.8 [34]. Based on Fig. 1, the pH value of Indian mackerel during storage at low-temperature fluctuates but tends to rise with pH values ranging between 5.8-7.4. The pH value is in the optimum pH range for the growth of spoilage bacteria that is in the range of 6.5-7.5 [35].

The initial pH value of control Indian mackerel on the first day of storage was lower (5.80) compared to the pH value of all Indian mackerel with solution soaking treatment (5.90). Based on the results of the examination, the ruku-ruku leaf stock solution used in the study had a slightly acidic pH value of 6.0, so that this value could affect the pH of Indian mackerel. Naturally, the pH in fish meat will decrease at the beginning of the storage period and then rise until the pH reaches base [36].

Chemical changes in fish meat begin with a decrease in pH that occurs due to the activity of the glucokinase enzymes in the body of the fish. These enzymes remodel glycogen into lactic acid which plays a role in reducing the pH of fish meat [37]. Over storage time, pH will increase again, this is due to proteins and their derivatives decomposed both by microbes and enzymatically into alkaline derivatives resulting in an increase in pH [38]. Decomposition of the protein will produce basic compounds such as ammonia, histamine, thiamine, and others [39]. An increase in pH in fish meat during storage indicates the activity of proteolytic enzymes that produce ammonia [40].

Storage of Indian mackerel at low-temperature by giving ruku-ruku leaf solution can slow the increase in pH. This is because at low-temperature the growth of bacteria becomes slow, so that protein overhaul lasts a long time, so the pH of fish is difficult to achieve basicity. According to Junianto [38], low-temperature conditions make the growth of bacteria in the body of the fish can be slowed down, so that the freshness of the fish is maintained longer.

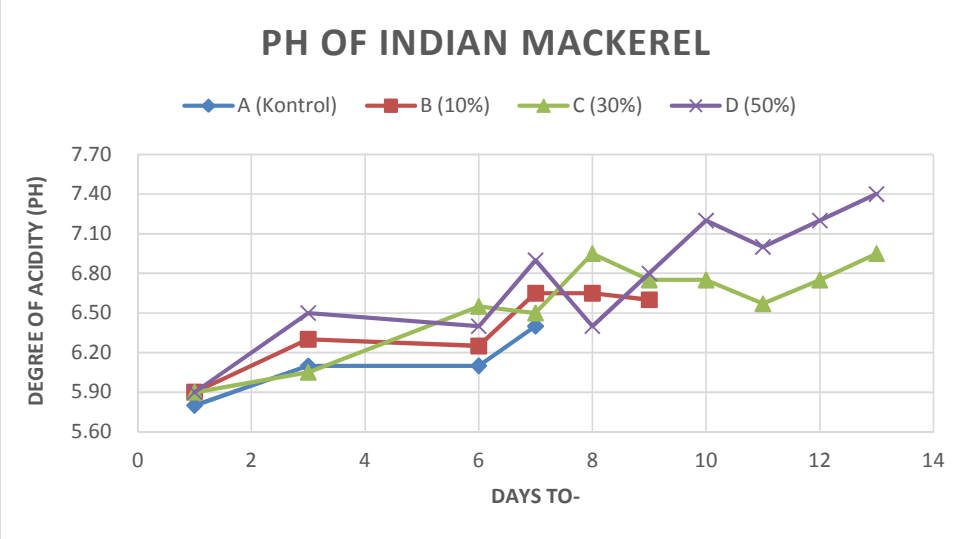

Fig. 1. Curves of Degree of Acidity (pH) in Indian Mackerel during Storage

In addition, it is also because the ruku-ruku leaf solution contains antimicrobial compounds that can inhibit bacterial growth in Indian mackerel so that the accumulation of ammonia can take place more slowly. Tannins contained in the ruku-ruku leaves can be one that affects the pH value of Indian mackerel. Tannins work by holding a hydrophobic complex with proteins, inactivating adhesin, enzymes and cell wall transport proteins that interfere with the growth of microorganisms [18].

If the growth of bacteria/microorganism disrupted the overhaul of proteins by bacteria will be less than the maximum, so that the pH of acidic fish meat can be maintained longer because of the lack of simple metabolites of protein (amino acids). A neutral to alkaline pH value is a good place for the growth of spoilage bacteria. The degree of acidity (pH) is related to amount of bacteria that grow, because as bacterial growth increases, it causes the overhaul of meat by bacterial activity which then produces basic compounds [41].

The pH value that has the best characteristics is the soaking treatment of 30% because it has a stable increase and decrease (fluctuation) pH and the storage limit has a pH value that is not too alkaline that is equal to 6.95, this pH value is in accordance with the research of Dewi et al. [27] that the use of ruku-ruku leaves with a concentration of 30% as an antibacterial in Indian mackerel during cold storage (4^{o}C) resulted in a pH value of 6.75 at the storage limit. According to Aprianti [42] degree of acidity Indian mackerel fresh between 6.9-7.2. A good source of nutrition can be obtained if the condition of the fish is fresh.

Indian mackerel treated with soaking of ruku-ruku leaf solution is still accepted until the 9th and 13th day, while Indian mackerel without soaking of the ruku-ruku leaf solution is accepted until the 7th day. As for Indian mackerel given 50% ruku-ruku leaf solution has a higher acidity (pH) (7.40) compared to Indian mackerel given 30% ruku-ruku leaf solution, only 6.95.

This shows that the addition of the concentration of the ruku-ruku leaf solution as an antibacterial to one point will inhibit bacterial growth but further addition will be the opposite because the organic material contained in Indian mackerel and the ruku-ruku leaf solution is used by bacteria to grow, giving rise to more base overhaul results. This is in line with Widiani's research [14] that the addition of extract concentrations as antibacterial to one point will inhibit bacterial growth but further addition will be the opposite.

3.4 Weight Loss

The results of observations of weight loss of Indian mackerel during low-temperature storage are presented in Fig. 2. The weight loss calculation is done to find out how much the weight loss of Indian mackerel from the beginning until the end of storage which has been soaked by a solution of ruku-ruku leaves during low-temperature storage. Weight loss is one of the physical changes caused by microorganisms that grow in a food [41].

Weight loss occurs because of the transpiration process, where the weight loss is greater at high temperature. With the loss of water in this transpiration process, the material is reduced in weight and water content. Much water is lost or evaporated from the material depending on the temperature and humidity of the environment [43,44].

Weight loss during storage of Indian mackerel at low-temperature occurs due to denaturation and autolysis processes. The denaturation process can occur due to heating or decreasing pH [45]. After undergoing denat-uration, the protein which was originally elastic will turn into hard, compact, and less elastic. Thus, the protein in Indian mackerel is no longer able to maintain the liquid it contains so that it drip.

Based on Fig. 2 shows that the weight loss in Indian mackerel during low-temperature storage has increased and decreased (fluctuating). The pattern of increasing and decreasing continues to occur in all treatments, either without soaking of ruku-ruku leaves solution or by soaking of ruku-ruku leaves solution, the highest weight loss results on the 7th day were found in Indian mackerel without treatment soaking ruku-ruku leaf solution (0%) of 6.59% compared to Indian mackerel with the treatment of ruku-ruku leaf solution (10%, 30%, 50%) which were only 6.25%, 6.25%, and 5.19%, respectively. This result is due to amount of bacteria found in Indian mackerel with soaking the ruku-ruku solution so that the reshuffle caused by bacteria and amount of drip that come out is also less. A small percentage loss in

weight indicates that protein still has the ability to bind water, so that free water in meat does not come out.

In addition, the lowest weight loss results on the 10th day were found in Indian mackerel with the soaking treatment of 30% ruku-ruku leaf solution at 5.94% while at 50% treatment the weight loss was at 6.67%. The final result of the storage limit on the best treatment (Indian mackerel treatment 30%) has a weight loss of 9.52%, this result is in accordance with research by Anggraeni et al. [46] that the catfish filet treated with the best guava leaf extract (20%) had a weight loss of 9.30%.

Increased weight loss during storage occurs because of the process of protein damage that causes the release of water bonds in fish meat. Damage to protein by enzymes from the fish's body and by bacteria will cause a reduction in the strength of the constituent meat in holding water [46]. Breakdown of proteins by enzymes derived from Indian mackerel into simpler components will cause the protein function as a binding of body fluids to decrease [47] and the fluid will come out of the tissue [41] so that weight loss occurs.

Then the increase in weight loss will cause an increase in spoilage bacterial populations. Overall the weight loss of Indian mackerel in each treatment is quite volatile except in the treatment of 10%, this is related to the water content of fish, where the water content and fat content of fish meat is very volatile, while the protein and mineral content is relatively constant [48].

The percentage of weight loss is in line with amount of bacteria found in Indian mackerel, ie the longer the shelf life, the weight loss will continue to increase and amount of bacteria will increase. The weight loss results in this study increased and decreased by 0.0%-12.00%. Increasing and decreasing the weight loss of Indian mackerel is in accordance with Anggraeni et al. [46] that the weight loss of the catfish filet given with guava leaf extract increased with increasing storage time and had a range of catfish filet weight loss in the range of 0.0%-12.60%.

The weight loss percentage for all treatments based on the acceptance deadline is in the range of 6.59%-9.52%. The relatively low weight loss percentage indicates that there is still a lot of protein in fish meat that has not been broken down by enzymes and still has the ability to bind water, so that free water in the water does not come out much. The amount of free water in meat will cause the growth of spoilage bacteria, due to activity water is one of the factors that influence the growth of spoilage bacteria [49].

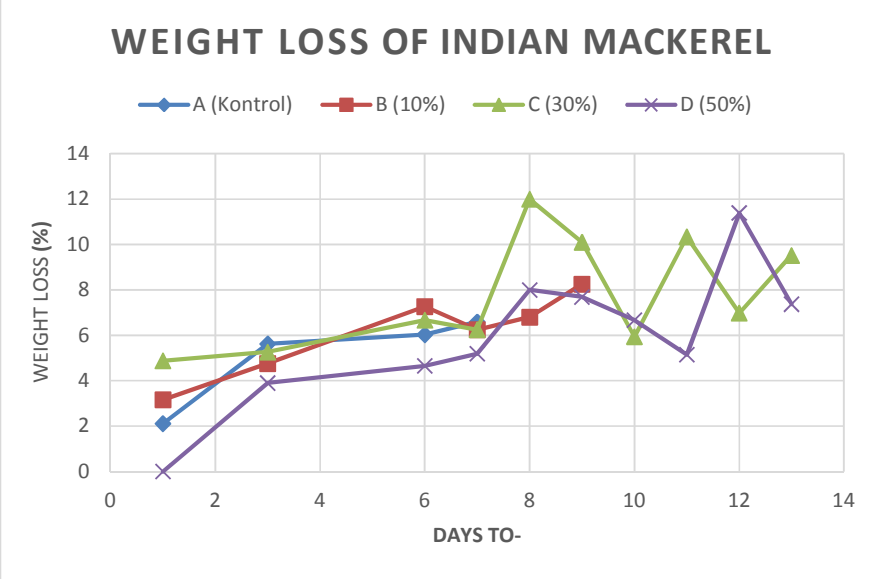

Fig. 2. Curves of Weight Loss in Indian Mackerel during Storage

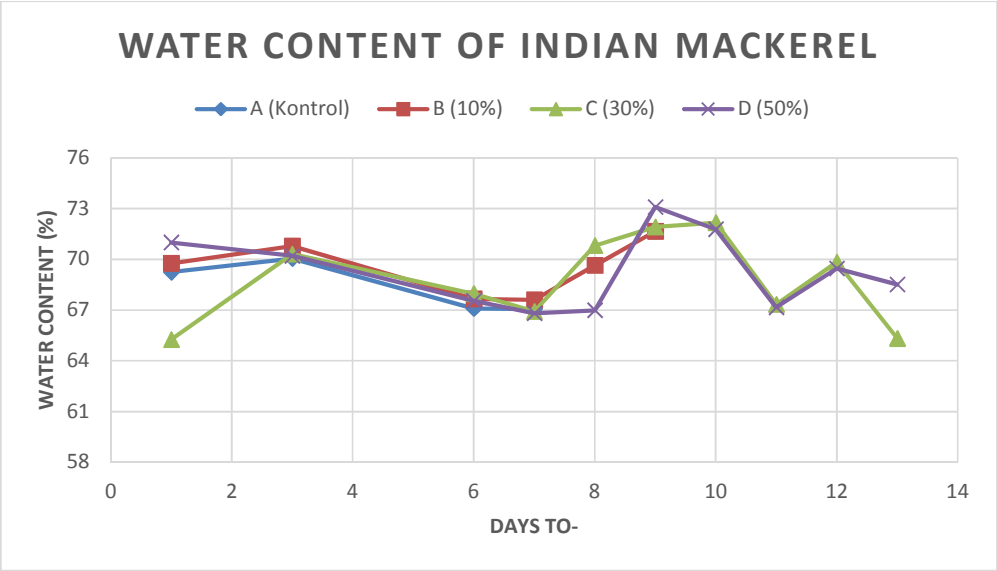

Fig. 3. Curves of Water Content in Indian Mackerel during Storage

The amount of drip (free water) that comes out of the fish will cause water levels to decrease and weight loss occurs [14]. Weight loss is closely related to water content, as stated Hadiwiyoto [41], that spoilage bacteria will break down simple metabolites into alkaline compounds which will increase the pH value of meat, due to the overhaul affect the weight loss and water content. Then if the water content decreases, the weight loss will increase.

3.5 Water Content

The results of observing Indian mackerel water content during low-temperature storage are presented in Fig. 3. Calculation of water content is carried out to find out how much water content is left from Indian mackerel from the beginning until the end of storage which has been soaked by a solution of ruku-ruku leaves during low-temperature storage. Water is an important component in food. All food ingredients contain different amounts of water, both animal and vegetable. The water content of materials shows the amount of water content per unit weight of material [50].

Fish is classified as a product that is prone to damage and spoilage (*highly perishable food*) because of its high protein and water content. Causes damage to fish includes high water content (70-80% of the weight of meat) that cause microorganisms are easy to grow and multiply [51].

Determination of water content is the most important analysis carried out in food processing and testing. Based on Kusnandar [52] water has an important role in the food system such as affecting freshness, stability, and food durability. The role of water in food is one of the factors that can affect metabolic activities such as enzymatic, microorganisms and chemicals that can affect the nutritional value of the product [53].

Based on Desniar et al. [54] the results of testing the composition of Indian mackerel (per 100 grams), the largest composition is water by 73.91%. Indian mackerel contains water high enough to be good for the growth of spoilage bacteria and microorganisms. Microbial growth and enzyme activity require certain water content. The more water content will increasingly allow microbes to grow and more active enzymes. Conversely, the less water content of a material will reduce microbial growth and enzyme activity [55].

Water content in food ingredients determines the durability of these foodstuffs. The lower the water content, the slower the growth of microorganisms so that food can last long [56].

However, if the fish's water content is high it can lead to relatively short shelf life. Water is a

necessity of all living things as well as bacteria. Bacteria need water for survival in addition to other nutritional components, so the higher the water content of a food, the faster the damage to food, the higher the bacterial activity [57].

High water content due to the presence of water that is not bound in the network of a substance or pure water with ordinary properties and full activity [56]. Besides, the increase in water content during storage is also caused by damage to protein, causing water to become bound to free water and increase water content [58]. Protein compounds contained in a substance containing constitutionally chemically bound water [59].

Generally, red meat has a low protein content, but higher water content. White flesh of fish has high protein content and low water content. Fish protein content is influenced by water content and fat content, that there is an inverse relationship between protein and water content in the edible part [47]. The lower water content in food, the higher the protein, carbohydrate, fat and mineral compounds [60].

Generally, the longer storage of food at low-temperature causes the water content to increase, but based on observations of Indian mackerel water content up to the 13th day, it is found to be quite volatile every day, this is reasonable because based on Irianto and Soesilo [48] states that the water content and fish fat content is very volatile, while the protein and mineral content is relatively constant. The water content in this study is still in accordance with SNI (01-26901-2006) regarding the determination of water content in fishery products, which recommends the water content in fishery products especially processed, is a maximum of 80% [61]. In addition, the water content of this study on the 6th day was 67.09-67.97%, this result is still in accordance with the research of Sari et al. [62] that the water content presto boiled Indian mackerel by leaves of ruku-ruku is the most high at 66.39% on the same day.

4. CONCLUSION

Indian mackerel with the soaking treatment of 30% ruku-ruku leaf solution is the most effective and best concentration during low-temperature storage (5-10^{o}C). Indian mackerel with soaking treatment of ruku-ruku leaf solution with a concentration of 30% is able to extend the shelf life to the 13th day with amount of bacteria of 6,90 × 10^{7} cfu/g, pH of 6.95, weight loss of 9.52%, and water content of 65.32%.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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