

# THE EFFICACY OF CHITOSAN FROM WINDU SHRIMP SHELLS AS A PRESERVATIVE OF FRESH KENYAR FISH IN THE FORM OF DARNE DURING STORAGE AT ROOM TEMPERATURE

Ni Nyoman Rupiasih<sup>1\*</sup>, Yosefa Yakunda Tandul<sup>1</sup>, Nyoman Wendri<sup>1</sup>, S. S. Jagtap<sup>2</sup>

<sup>1</sup>*Department of Physics, Faculty of Mathematics and Natural Sciences, Udayana University, Kampus Bukit Jimbaran, Badung, Bali, 80361, Indonesia*

<sup>2</sup>*Department of Physics, Haribhai V. Desai College, Pune-411002, MS, India*

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## ABSTRACT

This study is about the efficacy of chitosan from windu shrimp shells (*Panaeus monodon*) as a preservative for fresh fish in the form of darne (steak), namely fresh kenyar fish (*Sarda orientalis*) during storage at room temperature. The chitosan solution used are chitosan solution with concentration of 1%, 1.5%, 2%, and 2.5%. The fish samples which soaked in that solutions are named T1, T2, T3, and T4, respectively. The soaking time were 20, 40 and 60 minutes. As control were fish without treatment (C-0) and fish soaked in acetic acid (C-A). All samples stored at room temperature. The activity of chitosan as fish preservative was analyzed through pH and antimicrobial test. The pH test were made in the range of 0, 24, 48 and 72 h. The antimicrobial test was done on the storage period 24 h. The results showed that the greater the concentration of chitosan solution, the shorter the soaking time required to obtain the benefits of chitosan as an antimicrobial agent for fish preservation, especially kenyar fish in the form of darne. The fish that are still suitable for consumption for 24 hours of storage are fish that are soaked in a 2.5% (T4) chitosan solution at either 20, 40, or 60 minutes of soaking time.

**Keywords:** Chitosan; kenyar fish; darne; antimicrobial; preservatives.

## Introduction

Indonesia is a country that has abundant natural resources from land to sea. Indonesia is referred to as a maritime country, which is a country that has a sea area wider than the land. Therefore, Indonesia has a very diverse and abundant marine wealth. One of Indonesia's marine wealth is various types of fish. The sustainable potential of fish resources in Indonesian waters is estimated at 6.4 million tons/year.<sup>1</sup>

Fish is a nutritious food commodity and is widely consumed by the people of Indonesia, but fish storage has several obstacles, such as being easily damaged and decaying. Fish spoilage occurs as soon as the fish is caught and dies. Generally, fish decompose within 12-20 hours depending on the species, gear or method of fishing.<sup>2</sup> The process of spoilage in fish is caused by the activity of enzymes,

microorganisms, and oxidation in the fish body itself with changes such as a foul odor, stiff meat, faded eyes, and the presence of mucus on the gills and outer body.<sup>3</sup>

The preservation of fish aims to prevent spoilage bacteria from entering the fish. Fishermen and fish sellers usually use ice as a cooler in order to prolong the storage period of fish, but there are some people who use chemicals such as formalin which can be harmful to health. Research by Wardani and Mulasari (2016) on samples of salted fish sold in the Teluk Turtle Beach area, Cilacap Regency, showed that one sample was identified as positive for formalin.<sup>4</sup> Formalin is a substance that is prohibited to be used as a food preservative. The use of formalin in food can cause poisoning such as acute abdominal pain accompanied by vomiting, the onset of nervous system depression or

\*Corresponding author.

E-Mail: rupiasih@unud.ac.id

circulatory failure.<sup>2</sup> To avoid this, alternative antimicrobial materials are needed from natural ingredients that are harmless when consumed and can inhibit microbial growth in the product. These alternative preservatives must be safe for humans and easily available. One of them is chitosan, which is a natural compound that can be used to extend the durability of fresh products, because it has antimicrobial activity that can inhibit the growth of destructive microorganisms and has properties as a good coating for product interactions with the environment.<sup>5-7</sup>

Chitosan is a product of chitin deacetylation which is a long chain polymer of glucosamine 2-amino-2-deoxy- $\beta$ -(1-4)-D-Glucose, having the molecular formula  $[C_6H_{11}NO_4]_n$ . Chitosan is a biopolymer that is unique in that in acidic solution it has cation characteristics and is positively charged while in alkaline solution it precipitates.<sup>5, 7</sup> Meanwhile, chitin is the main component of the exoskeleton of invertebrates, crustaceans and insects that functions as a supporting and protective component. Chitin is a polysaccharide that has a high molecular weight and is a linear polymer with other names 2-acetamide-2-deoxy- $\beta$ -(1-4)-D-glucose or N-Acetyl-D-Glucosamine.<sup>5, 8-9</sup>

Chitosan can be used in various fields of life such as biomedical, cosmetic, biotechnology, agriculture and food. Chitosan and its modifications have also been widely recognized as natural preservatives, biodegradable, and as anti-bacterial which is stronger than lactic acid. Chitosan has a strong positive charge that can bind to a negative charge as a detoxifier and inhibit microbial growth.<sup>5-6, 10-11</sup>

Several studies have been conducted regarding the use of chitosan as an antimicrobial in fish. Wittriansyah<sup>12</sup> have reported the application of chitosan from sea urchin (*Emerita* sp.) as an alternative preservative in mullet (*Mugil cephalus*). Silvia<sup>1</sup> have also reported the use of chitosan from crab shells (*Portonius sanguinolentus* L.) as a preservative for mackerel (*Rastrelliger* sp.) and catfish (*Clarias batrachus*). The results showed that fish preservation by

soaking using 1.5% chitosan solution was the best solution concentration and could extend the shelf life of fish for 6 hours, while fish preservation by spraying obtained the best solution concentration of 2.5%, which could extend the shelf life of fish up to 4 hours.

This study reported the efficacy of chitosan from windu shrimp shells (*Panaeus monodon*) as a preservative for fresh fish in the form of darne, namely fresh kenyar fish (*Sarda orientalis*) during storage at room temperature. Kenyar fish are pelagic fish belonging to the Scombridae family. Kenyar fish is a fishery commodity that is classified as economically important, one of which is as a raw material for the fish canning industry.

Today, local traditional fishermen use ice as temporary storage for their catch while they are still in the boat. They keep the fish intact. From this background it is important to find other alternatives for temporary storage of fish and the fish has been cut into steaks to save space. For this, it chosen chitosan as an alternative of fish preservation. The results of this study are expected to be used as a reference in the use of chitosan especially chitosan extracted from windu shrimp shells as a preservative for kenyar fish caught by local traditional fishermen, Jimbaran Bali.

## Methods

The materials used were chitosan powder from windu shrimp shells obtained from Chemical Lab Universitas Sumatra Utara, kenyar fish, acetic acid, sterile water, and nutrient agar. The concentration of chitosan solution used to soak the fish were 1%, 1.5%, 2%, and 2.5%. The volume of chitosan solution and the mass of darne fish kenyar for each sample were 500 ml and 500 g. Each sample is named T1, T2, T3, and T4. As a control was a sample of darne kenyar fish soaked in acetic acid, which was named C-A and a control that was not soaked in either solution (no treatment) named C-0. Soaking time was 20, 40 and 60 minutes; Observation times were 0, 24, 48, and 72 hours. Parameters tested were pH and antimicrobial test.

**a. pH observation**

The pH test was carried out using litmus paper to determine the level of acidity or alkalinity of the control sample as well as by treatment.<sup>13</sup> The steps were five (5) g of kenyar fish that had been soaked in a chitosan solution, homogenized in 10 mL of distilled water using a mortar for 1 minute. The kenyar fish mixture was poured into a beaker, then the pH was measured using litmus paper.

**b. Antimicrobial test**

The antimicrobial test is done by total plate count (TPC) method. The total plate count was observed by counting the number of colonies that grew on the agar which had been mixed with the diluted sample.<sup>14</sup> Sample testing was carried out in triplicate; and one photo of each antimicrobial test is shown.

The steps are 10 g sample and 90 mL sterile Butterfiel's Phosphate Buffered solution is homogenized. This was called the first dilution sample. One (1) mL of the first dilution sample was pipetted into the Butterfiel phosphate buffer solution to obtain

a 10<sup>-2</sup> dilution. This was called the second dilution sample. Next, a dilution (10<sup>-3</sup>) was made by taking 1 mL of the second dilution sample and homogenized with 9 ml of Butterfiel phosphate buffer solution. This were called the third dilution sample. The same process was continued until the dilutions of 10<sup>-4</sup> and 10<sup>-5</sup> were obtained; they called the fourth and fifth dilution samples. Each 1 mL of the fourth and fifth dilution samples were poured into sterile petri dish; then was added 12-15 mL of agar and shaken to homogenized. Those samples were incubated at 35 °C for 24 hours. The colonies were counted which had the number of colonies from 25 to 250; then multiplied by the dilution factor.

**Results and Discussion**

**a. Determiation of the degree of acidity (pH)**

The average pH obtained in those samples (T1, T2, T3, T4, and C-A) are written in Table 1. The average pH for sample C-0 are listed in Table 2.

**Table 1.** The average pH of samples T1, T2, T3, and T4, and control samples C-A, where the soaking time of 20, 40, and 60 minutes and storage time (observation) 0, 24, 48, and 72 hours

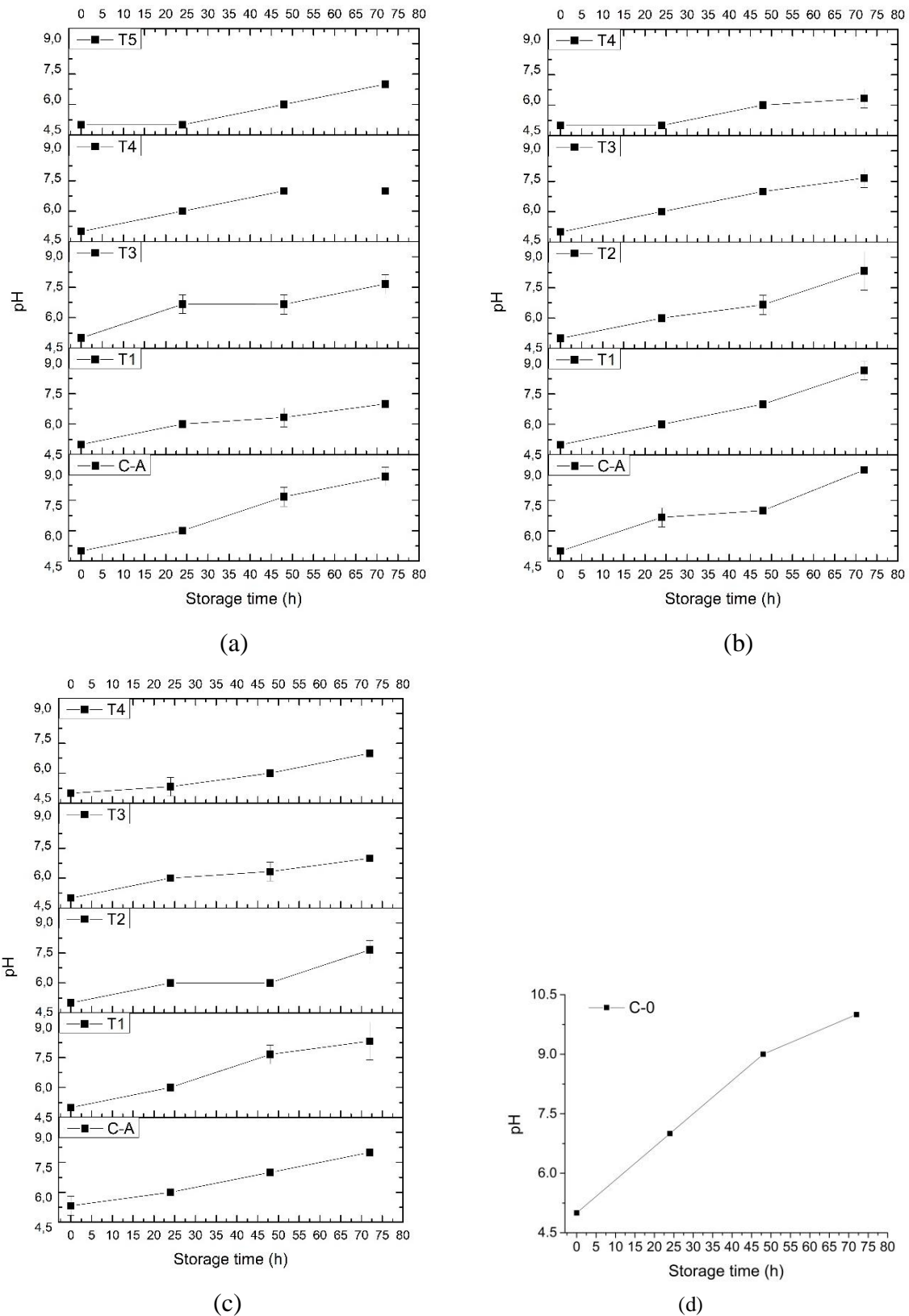
Soaking time (minutes)	pH											
	20				40				60			
Storage time (h)	0	24	48	72	0	24	48	72	0	24	48	72
Control C-A	5.00±0.00	6.00±0.00	7.67±0.47	8.66±0.47	5.00±0.00	6.66±0.47	7.00±0.00	9±0.00	5.33±0.47	6.00±0.00	7.00±0.00	8.00±0.00
T1	5.00±0.00	6.00±0.00	6.33±0.47	7.00±0.00	5.00±0.00	6.00±0.00	7.00±0.00	8.66±0.47	5.00±0.00	6.00±0.00	7.66±0.47	8.33±0.94
T2	5.00±0.00	6.67±0.47	6.66±0.47	7.66±0.47	5.00±0.00	6.00±0.00	6.66±0.47	8.33±0.94	5.00±0.00	6.00±0.00	6.00±0.00	7.66±0.47
T3	5.00±0.00	6.00±0.00	7.00±0.00	7.00±0.00	5.00±0.00	6.00±0.00	7.00±0.00	7.66±0.47	5.00±0.00	6.00±0.00	6.33±0.47	7.00±0.00
T4	5.00±0.00	5.00±0.00	6.00±0.00	7.00±0.00	5.00±0.00	5.00±0.00	6.00±0.00	6.33±0.47	5.00±0.00	5.33±0.47	6.00±0.00	7.00±0.00

**Table 2.** The average pH of control sample without treatment (C-0)

Storage time (h)	0	24	48	72
pH	5.00±0.00	7.00±0.00	9.00±0.00	10.00±0.00

The data in Table 1 and Table 2 can be graphed between soaking time, concentration of chitosan solution, and storage time on the

pH of each sample, Controls C-0 and C-A, and samples with treatments T1, T2, T3, and T4, as shown in Figure 1.



**Figure 1.** Graph of the average pH vs storage time of samples soaked in chitosan solution (T1, T2, T3, and T4) and sample soaked in acetic acid (C-A) with soaking time: a) 20, b) 40, and c) 60 minutes, and d) the average pH of sample C-0.

Figure 1 shows that in most of the samples, the pH increased in the 24-72 hours storage

period. A good pH for preserved fish ranges from 2.0 to 5.5, while pH of 6.0 to 8.0 is a

good pH for the growth of microorganisms.<sup>15</sup> In the T4 sample, namely the sample soaked in 2.5% chitosan solution, the pH of the sample with storage time of up to 24 hours was still 5, both for the soaking time of 20, 40 and 60 minutes. In this case the fish samples are good for consumption. While in other samples, the average pH of the sample increased by 1 for the addition of 24 hours of observation time, even the control sample (C-A) showed the highest pH, that was 8 for 72 hours of storage. So, fish soaked in 2.5% chitosan solution could maintain their pH until 24 hours, for either 20, 40, or 60 minutes of soaking. From these observations, it was

found that kenyar fish that were still good for consumption were kenyar fish by soaking in 2.5% chitosan solution with a storage time of up to 24 hours. It can be seen that the pH of kenyar fish that were not soaked with chitosan or acetic acid solution showed a significant increase in pH, even at 24 hours the pH had increased to 7.

**b. Antimicrobial test**

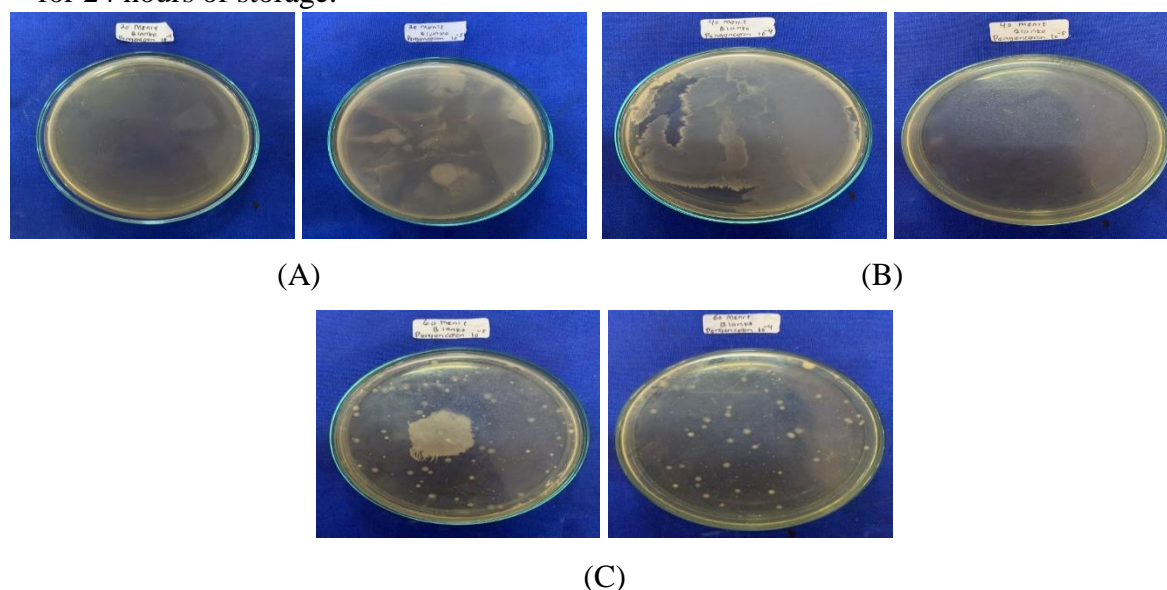
The results of antimicrobial test, it obtained the number of microbes in samples C-A, T1, T2, T3, and T4 as shown in Table 3. These results are also accompanied by photos as shown in Figure 2-6.

**Table 3.** The total number of microbes (CFU/mL) of each sample, which was obtained by the total plate count method

Sample (storage for 24 h)	The number of microbes during storage (CFU/ml)		
	Soaking for 20 minutes	Soaking for 40 minutes	Soaking for 60 minutes
Control (C-A)	$\geq 250 \times 10^5$	$\geq 250 \times 10^5$	$247.1 \times 10^5$
T1	$129 \times 10^5$	$\geq 250 \times 10^5$	$\geq 250 \times 10^5$
T2	$\geq 250 \times 10^5$	$\geq 250 \times 10^5$	<b>*Negative</b>
T3	$\geq 250 \times 10^5$	<b>*Negative</b>	$\geq 250 \times 10^5$
T4	<b>*Negative</b>	$\geq 250 \times 10^5$	$107 \times 10^5$

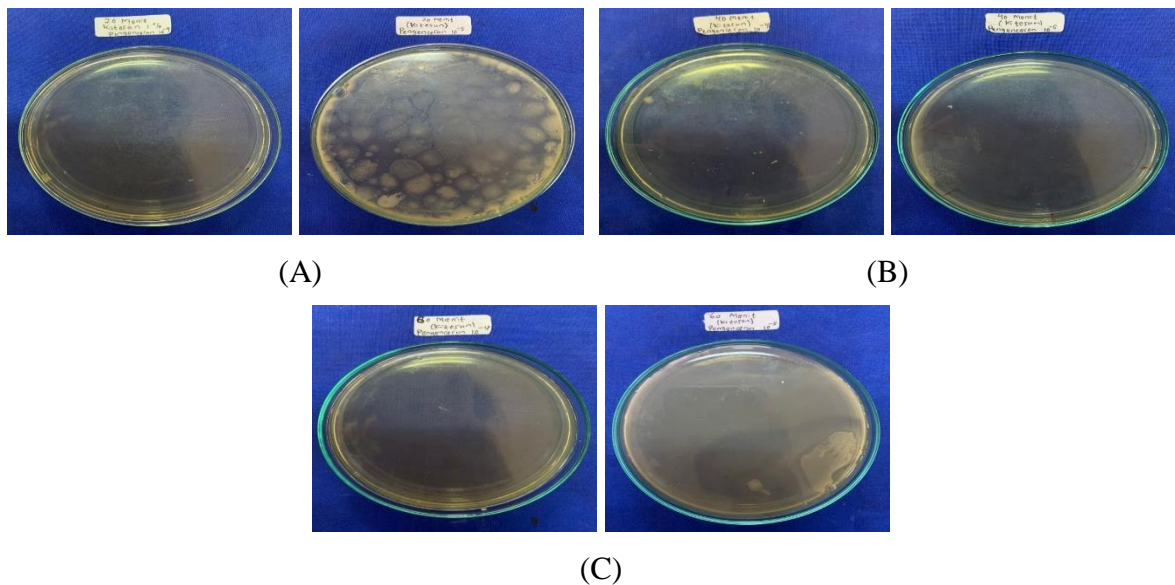
\*Negative = no microbes present.

a) The photos of antimicrobial test of sample C-A with soaking times: 20, 40, and 60 minutes, for 24 hours of storage.



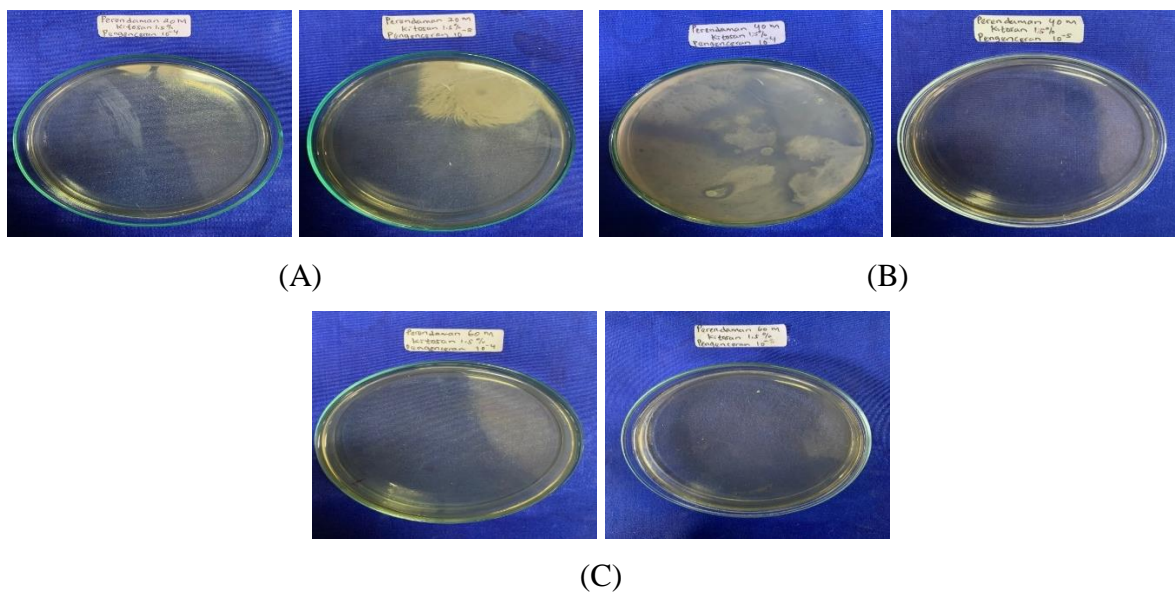
**Figure 2.** The photos of antimicrobial test of sample C-A for 24 hours of storage with soaking times: (A) 20, (B) 40, and (C) 60 minutes.

- b) The photos of antimicrobial test of sample T1 with soaking times: 20, 40, and 60 minutes, for 24 hours of storage.



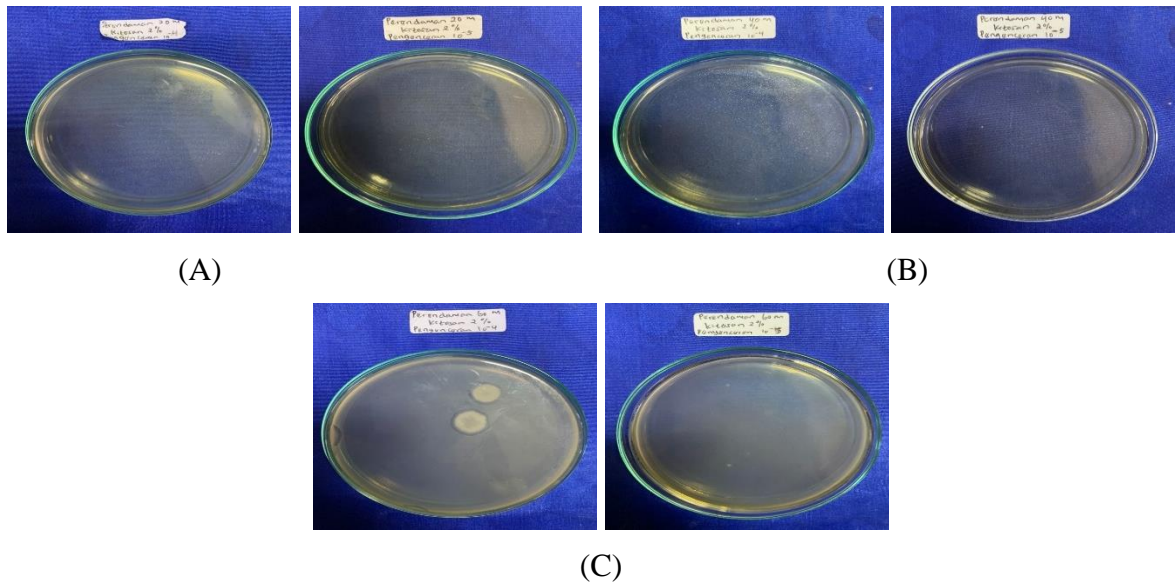
**Figure 3.** The photos of antimicrobial test of sample T1 for 24 hours of storage with soaking times: (A) 20, (B) 40, and (C) 60 minutes.

- c) The photos of antimicrobial test of sample T2 with soaking times: 20, 40, and 60 minutes, for 24 hours of storage.



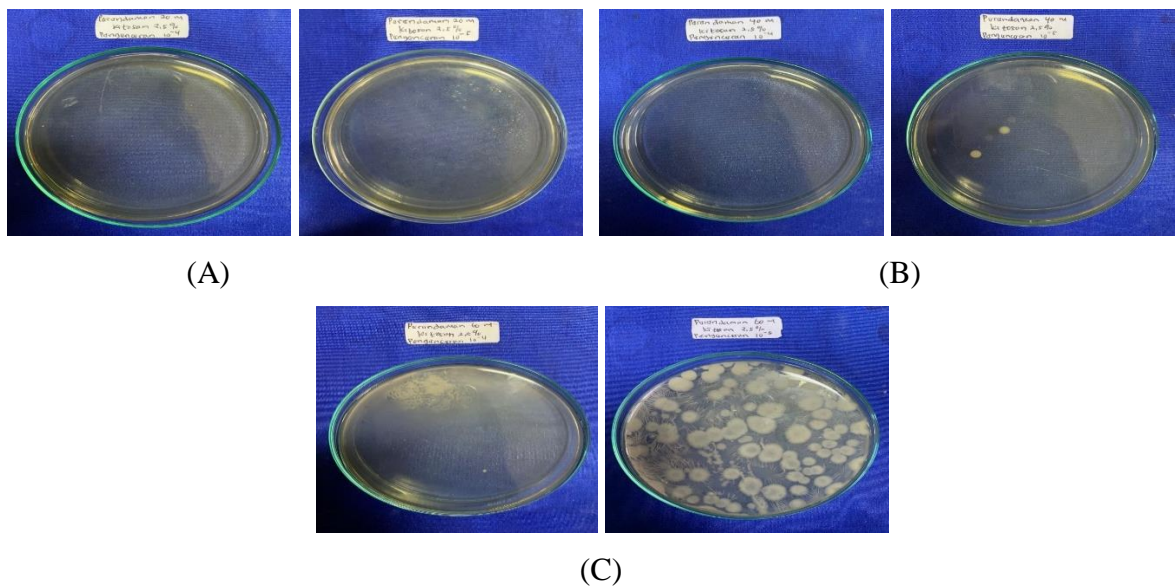
**Figure 4.** The photos of antimicrobial test of sample T2 for 24 hours of storage with soaking times: (A) 20, (B) 40, and (C) 60 minutes.

- d) The photos of antimicrobial test of sample T3 with soaking times: 20, 40, and 60 minutes, for 24 hours of storage



**Figure 5.** The photos of antimicrobial test of sample T3 for 24 hours of storage with soaking times: (A) 20, (B) 40, and (C) 60 minutes.

e) The photos of antimicrobial test of sample T4 with soaking times: 20, 40, and 60 minutes, for 24 hours of storage.



**Figure 6.** The photos of antimicrobial test of sample T4 for 24 hours of storage with soaking times: (A) 20, (B) 40, and (C) 60 minutes.

The data from Table 3 shows that soaking kenyar fish in a chitosan solution with a certain concentration and soaking time can inhibit microbial growth. The soaking kenyar fish in 1.5% chitosan solution (T2) with 60 minutes of soaking, in 2% chitosan solution (T3) with 40 minutes of soaking and in 2.5% chitosan solution (T4) with 20 minutes of soaking, can inhibit the growth of microorganisms. It is highlight with grey

colour in the value. So the greater the concentration of chitosan solution, the shorter the soaking time required to obtain the benefits of chitosan as an antimicrobial agent for fish preservation, especially kenyar fish caught by local fishermen in the form of darne.

The results of this study are in accordance with several studies that have been reported as follows. Jayawardana and Ahmad Afandi

reported that fish cilok (a fish ball shaped food) coated with 1.5% chitosan solution stand longer than fish cilok without or added with 1.5% chitosan solution up to fourth day preservation in a room temperature.<sup>16</sup> Toyne et al (2015) reported that the cork fish minced meat (*Channa striata*) coated with chitosan solution of concentration of 3% able to avoid hydrolysis and inhibit bacterial activity, so minced cork meat can last up to 3 days.<sup>17</sup> Venesya Souhoka et al (2021) reported that the smoked tuna coated by chitosan solution a concentration of 2% can stand with a shelf life until 4 days at room temperature.<sup>18</sup> These studies are basically about the application of chitosan as a fish preservative where different types of fish and processed types are used, the forms of treatment given are also different, and also with different results. However, in general it can be said that chitosan can extend the shelf life of fish and its products at room temperature. So our research can enrich information and references on the use of chitosan as a preservative for fresh marine fish, namely kenyar fish in the form of darne at room temperature.

## Conclusion

This study has demonstrated one of the applications of chitosan namely as a preservative for fresh fish, especially kenyar fish in the form of darne for storage at room temperature. It obtained that the greater the concentration of chitosan solution, the shorter the soaking time required to obtain the benefits of chitosan as an antimicrobial agent for fish preservation, especially kenyar fish caught by local fishermen in the form of darne. Fish that are still suitable for consumption for 24 hours of storage are fish that are soaked in a 2.5% (T4) chitosan solution at either 20, 40, or 60 minutes of soaking time.

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