Use of Sargassum polycystum ethanol extract as antibacterial for increasing shelf life tilapia fillet (Oreochromis niloticus) stored in chilling temperature

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Abstract. Sargassum polycystum is a seaweed that has a chemical compound that can be used as an antibacterial. Tilapia fillet is one of the fish product which is known as perishable food. The purpose of this study was to see the effect of S. polycystum ethanol extract in resisting the deterioration rate of tilapia fillet quality. The tilapia fillet was given three treatments that was soaked with 1% S. polycystum ethanol extract, positive control (washed with 10 ppm chlorine water), and negative control (water) was then stored for 10 days at a temperature of 4°C. The results of the study found that tilapia fillets that are soaked with S. polycystum ethanol extract solution can maintain the organoleptic value. TPC of tilapia fillet soaked with S. polycystum ethanol extract was better than control. Chemical test results (pH and TVB) showed the fillet that was soaked with S. polycystum ethanol extract had a better value than the control. Treatment with S. polycystum ethanol extract acts as an antibacterial compound similar to the positive control, but better than the negative control. This can be used in extending the shelf life of tilapia fillets in low temperatures by two days longer than negative control.

Keywords: antibacteria, S. polycystum, shelf life, tilapia fillet

1. Introduction

Some types of seaweed such as Sargassum sp, Caulerpa sp., Padina sp. and Gelidium sp. contain antibacterial compounds [1]. Sargassum polycystum is a seaweed that contains chemical compounds and can be used as antibacterial [2]. Antibacterial compounds derived from S. polycystum marine algae have the prospect of being a natural material for fishery products. Nila or tilapia as one of fishery product that is easily damaged. This damage occurs in biochemistry and microbiology. The existence of these processes has been felt to hamper the marketing efforts of fishery products and not infrequently causes large losses [3]. Therefore, it is necessary to make an effort to increase the shelf life of fishery products through processing and preservation, one of which is by cold storage. However, cold storage still has limitations, namely the relatively short shelf life of meat [4]. The main cause of damage to fish during cold storage is the activity and growth of psychotropic bacteria [5]. Therefore, it is necessary to seek control to increase the shelf life of fishery products during cold storage such as by using seaweed extract. The purpose of this study was to find the effect of S. polycystum extract in resisting the deterioration rate of the quality of tilapia fillets during cold storage.
2. Materials and Methods

2.1. Materials and tools
The material used in this study consisted of S. polycystum and indigo fillets, ethanol, paper disks, Staphylococcus aureus and Pseudomonas aeruginosa isolates, Trypticase Soy Broth (TSB) and Tryptic Soy Agar (TSA). The equipment used includes: orbital shaker, rotary vacuum evaporator, aerator, blender, scales, and incubator.

2.1.1. Identification of S. polycystum seaweed. Sampling was done randomly S. polycystum (random sampling) in the waters of the Gulf Banten Serang regency of Banten province, by taking seaweed cut then washed with sea water, drained by way of cooling it with wind without direct sun exposure and put into a clean sack. Identification of samples of S. polycystum seaweed was carried out by studying morphology and then matched with literature.

2.1.2. Polycystum S. extraction. The sample of S. polycystum used in this study was taken from the bay waters of Banten Province Attack District. Sampling was done by exploring the island and taking thallus parts using scissors. After collection, then the thallus was put in a clean bag. S. polycystum obtained was then drained and then winded without direct sunlight contact. Before extracting, S. polycystum was dried by wind without exposure to direct sunlight. Furthermore, extraction of S. polycystum was done using the method from previous research [6]. S. polycystum which was dried then blended until it becomes simplicia then simplicia was given 96% ethanol (1:4) then macerated with orbital shaker for 2x24 hours. The extract obtained was then evaporated at a speed of 60 rpm and temperature 40°C, then extract aerated with an aerator until crude extract was obtained in the form of paste.

2.1.3. Antibacterial activity testing. Testing the antibacterial activity of S. polycystum extract against Staphylococcus aureus and Pseudomonas aeruginosa was done using diffusion method. The stages in this method included the preparation stage and the testing phase. The preparation stage included making TSA media, and inoculating test bacteria Staphylococcus aureus and Pseudomonas aeruginosa grown on TSB media to TSA media using the swab method. The testing phase is a sterile paperdisk which drops 20 µl/mL. S. polycystum extracts with the concentration of each paperdisk at 20 µg/mL, 50 µg/mL, 100 µg/mL, 150 µg/mL. Then the paperdisk is attached to the surface of the TSA media. Subsequently it was incubated at a temperature 35°C for 24 hours. After incubation, the inhibition zone (clear zone) is measured using a ruler.

2.1.4. Storage of fillets at cold temperatures with treatment. Storage of tilapia fillets at cold temperatures were given three treatments. Tilapia (200-350 g) was obtained from the freshwater fish hatchery CV. Jalil Santing, Pasar Minggu, South Jakarta. The first treatment, S. polycystum extract was dissolved in distilled water as solution for 1% of concentration subsequently tilapia fillets were soaked for 30 minutes in it. The second treatment for tilapia fillets were washed with chlorine water with a concentration of 10 ppm and the third treatment for tilapia fillets were washed with plain water as a control, then stored in cold temperatures (4°C) for 10 days with an observation interval every 2 days. Parameters observed included: pH, Total Plate Count (TPC) [7], Total Volatile Base-Nitrogen (TVB-N) [8], and organoleptic [9].

3. Results and Discussion

3.1. Identification of S. polycystum seaweed
Extraction of S. polycystum seaweed is located in the waters of Banten Bay, Serang Regency, Banten Province, precisely on the Island of Lima (figure 1). Sampling is done by combing the beach area of Pulau Lima. Based on the geographical location, the sampling locations were at coordinates 06 ° 0'5 .6376 "South Latitude and 106 ° 9'15.3684" East Longitude. Polycystum S. seaweed can be found at a depth of 1-2m attached to the sandy substrate. S. polycystum seaweed which is found in the waters of Lima Island Serang Regency, Banten Province has the characteristics of a small spiny cylindrical thallus, small leaves, long oval, jagged edges, and pointed edges and has air bubbles, identification of S. polycystum seaweed seen from the key of determination and literature study.
3.2. Yield and antibacterial activity of *S. polycystum* extract

The yield resulting from the extraction of *S. polycystum* using 96% ethanol was 3.34%. The percentage of yield produced from macroalgae extract using ethanol solvent ranged from 2-3% [10]. The results of testing the antibacterial activity of *S. polycystum* extract on *S. aureus* and *P. aeruginosa* are presented in table 1.

### Table 1. Inhibition zone of *S. polycystum* extract (mm).

| Time incubated | Test Bacterial | Concentration (μg/mL) | Control (+) | Control (-) |
|----------------|----------------|-----------------------|-------------|-------------|
|                |                | 25 | 50 | 100 | 150 |               |             |
| 24 Hours       | *S. aureus*    | 5.12 | 5.14 | 6.08 | 6.12 | 14.18 | (negative) |
|                | *P. aeruginosa*| 5.02 | 5.12 | 5.18 | 6.18 | 10.02 | (negative) |
| 48 Hours       | *S. aureus*    | 5.02 | 5.10 | 5.16 | 5.18 | 14.12 | (negative) |
|                | *P. aeruginosa*| 4.19 | 5.12 | 5.16 | 6.12 | 9.04  | (negative) |
| 72 Hours       | *S. aureus*    | 4.16 | 5.10 | 5.14 | 5.18 | 12.10 | (negative) |
|                | *P. aeruginosa*| 4.12 | 5.04 | 5.14 | 5.18 | 7.12  | (negative) |

The area of inhibitory zone formed by testing the antibacterial activity of *S. polycystum* extract against *S. aureus* is wider than the inhibitory zone area of *P. aeruginosa*. This is possible because Gram positive bacteria are more susceptible to some marine algae extracts than Gram negative bacteria [11-13]. This is caused by differences in the structure and constituent of the cell wall [14]. Secondary metabolite compounds contained in marine algae that have the potential to be antibacterial are phenols, peptides and terpenes [15]. The brown algae class contains terpene compounds and phenols which have antibacterial and antioxidant activity [16].

3.3. Bacterial residue testing (TPC) in different washing treatments

The results of residual washing tests on tilapia fillets with 1% *S. polycystum* extract treatment, 10 ppm chlorine, and control during cold storage can be seen in figure 2. Total Plate Count (TPC) is one of the parameters in the process of fish quality decline. Due to the loss of the fish's natural defense system after death, bacteria can easily enter and attack fish meat through the skin, gills and digestive tract [17].
It was seen that washing with 1% S. extract gave a decrease in the number of bacteria by 35% of the treatment before washing. This is because *S. polycystum* extract contains bioactive compounds that have the ability to inhibit bacterial growth, such as phenol compounds that can interfere with the bacterial cell membrane. Some marine algae from the brown algae class contain terpene compounds and phenols which have antibacterial and antioxidant activity [16].

3.4. Quality test for tilapia fillets

3.4.1. Organoleptic testing. Organoleptic testing uses a *score sheet* according to [9] based on three parameters, namely appearance, smell and texture. The organoleptic value of indigo fillets can be seen in table 2. The data in table 2 shows that the organoleptic value of tilapia fillets for all treatments decreased with the length of storage. The results of organoleptic tests for appearance parameters showed that the organoleptic value of the appearance for the fillet not given treatment could not be accepted until the 6th day of storage with an organoleptic value of 6.1 with the appearance of cream, dull, lateral lines sliced brown. The treated tilapia fillet which was washed with 10 ppm chlorine could not be received on the 8th day of storage with an organoleptic value of 6.1 with a fillet appearance, a creamy incision, less brilliant, a slightly brown line of literalis. The tilapia fillet treated with immersion extract was not acceptable on the 8th day of storage with organoleptic value 6.1 equal to the fillet with the treatment washed with 10 ppm chlorine, where the fillet was brownish fleshted, less brilliant, with a slightly brown line. The use of *S. polycystum* extract has an effect on the appearance of tilapia fillets, namely brownish meat incisions. The brown color of the tilapia fillet is caused by the dominant color of *S. polycystum* which belongs to the class of brown algae which has brown pigments in the form of fucoxanthin, so that the *S. polycystum* extract is brownish [15].

Odor parameter test results showed that the organoleptic value of odor continued to decrease along with the length of storage. In the indigo fillet that was not treated, it could not be accepted until the 6th day with an organoleptic value of 6.2 where the fillet odor began to be neutral. Tilapia fillet treated with 10 ppm of chlorine water was not acceptable to panelists until the 8th day with an organoleptic value of 6.2. Fillets which were given extract treatment were not acceptable to panelists until the 8th day with an organoleptic value of 6.25. The specification of indigo fillet odor is fresh odor, specific of freshwater fish and slightly muddy in odor [9]. Odor in indigo fillet is due to the presence of volatile compounds that smell like ammonia, resulting in low organoleptic scores [18].
Test results for texture parameters during storage decreased. Tilapia fillet in the control treatment began to be unacceptable to panelists until day 4 with organoleptic values of 6.8 where the fillet texture was rather compact and less elastic in the fillet of tilapia given extract treatment, it was not accepted until day 8 with an organoleptic value of 6.0. And the fillet of tilapia treated with 10 ppm of chlorine began to not be accepted until day 8 with an organoleptic value of 6.0. The specifications of tilapia fillets after being stored for 10 days at cold temperatures have a texture that starts to soften, is less compact and less elastic [9]. The overall organoleptic of tilapia fillet during storage is a decreased organoleptic value along with the length of storage. The overall organoleptic value on day 6 still revolved around the organoleptic condition of fresh frozen tilapia fillet products, namely 7. Specifications of indigo fillet which are whole, clean, milky white incisions, less bright colorline, fresh odor, freshwater fish specifications, slightly muddy smell, solid, compact and elastic texture [9]. Tilapia fillet which were given \textit{S. polycystum} extract were still suitable for consumption until the 8th day storage because it had an organoleptic value ranging from 7.

### Table 2. Organoleptic value of tilapia fillet.

| Parameter | Storage time (days) | SNI |
|-----------|---------------------|-----|
|           | 0  | 2  | 4  | 6  | 8  | 10 |
| Appearance|    |    |    |    |    |    |
| Extract   | 8.27 | 7.89 | 7.61 | 7.14 | 6.11 | 5.56 |
| Chlorine  | 8.40 | 8.06 | 7.66 | 7.20 | 6.13 | 5.59 |
| Control   | 8.22 | 7.72 | 7.02 | 6.14 | 5.36 | 4.57 |
| Smell     |    |    |    |    |    |    |
| Extract   | 8.28 | 7.92 | 7.63 | 7.09 | 6.25 | 5.64 |
| Chlorine  | 8.43 | 8.18 | 7.74 | 7.16 | 6.27 | 5.81 |
| Control   | 8.23 | 7.77 | 7.01 | 6.21 | 5.53 | 4.69 |
| Texture   |    |    |    |    |    |    |
| Extract   | 8.23 | 7.81 | 7.51 | 7.09 | 6.01 | 5.55 |
| Chlorine  | 8.35 | 7.98 | 7.53 | 7.10 | 6.04 | 5.65 |
| Control   | 8.20 | 7.69 | 6.85 | 6.09 | 5.22 | 4.49 |

#### 3.4.2. Total plate count (TPC).

TPC results can be seen in figure 3. The number of bacteria at the beginning of fillet storage in all treatments is increasing along with the length of storage. Increased total bacterial content in fillet tilapia is because fish meat is a medium suitable for bacterial growth which causes bacteria to grow [19]. On the other hand, meat also contains high protein, so the process of damage to meat by microbial activity during storage results in the decomposition of chemical compounds in meat [20]. In figure 3 it can be seen that the results of TPC testing on the number of bacteria in all treatments on day 0 to 10 days have not exceeded the standard limit where tilapia fillets are still suitable for consumption. The TPC of the tilapia fillet control was higher than that of the fillet given \textit{S. polycystum} extract (see figure 2).

This is because the effectiveness of \textit{S. polycystum} can inhibit bacterial growth, because it contains bioactive compounds that have the ability to inhibit bacterial growth, such as phenol compounds that can interfere with the bacterial cell membrane. Some marine algae from the brown algae class contain terpene compounds and phenols which have antibacterial and antioxidant activity [16].
3.4.3. Testing the pH of fillets. The results of pH test for fillets during cold storage can be seen in figure 4. Based on figure 4, it can be seen that the pH falls on the second day of storage, but the 6th to 10th day increases. Previous research showed fluctuations in the pH value of tilapia for each treatment at low temperature storage for 10 days [19]. This is possible because of the little glycogen reserves in the fish.

![Figure 4. The pH value during storage, = extract, = chlorine, = control, = acceptance limit.](image)

During cooling and freezing the pH of the meat will change the pH value of fish meat will drop from about 7 to 6.3 then rise again during low temperature storage [4]. This change occurs at the initial stage of cooling or freezing, the pH of the fish meat will go down then it will rise again. The occurrence of a decrease and increase in pH is much associated with the physiological state of fish meat. The increase in pH may also be caused by psychrophilic bacteria which cause the formation of more volatile bases.

3.4.4. Total Volatile Base-Nitrogen Content (TVB-N). The results of TVB-N tilapia fillet testing during cold storage can be seen in figure 5. Tilapia TVB-N content increased in proportion to storage time. The increase in the content of TVB-N tilapia fillets with extract treatment was slower when compared with no extract / control. This is possible because of the presence of antibacterial compounds in the extract. So that it can inhibit the activity of decomposing bacteria found on the tilapia fillets without extract. According to [21] in [22] an increase in TVBN content in fish meat during storage due to the degradation of proteins and their derivatives by microorganisms that produce volatile bases such as trimethylamine (TMA), ammonia, and H$_2$S. Limits for consumer acceptance of fresh fish content of 30 mgN/100 g fish meat [23]. Fillets of tilapia without treatment (control) shows that it has exceeded the limit of consumer acceptance on day 10 with a TVB value of 30.99 mgN/100 g. The treatment of extracts and chlorine was still below the consumer acceptance limit until the 10th day with TVB values in the extract treatment at 27, 26 mgN/100 g, and in chlorine treatment 24 mgN/100 g. This shows that the extract treatment can slow down the increase in TVB value in the tilapia fillet.

![Figure 5. TVB-N during cold storage, = extract, = chlorine, = control, = acceptance limit.](image)
Figure 5. The TVB value of tilapia fillet during cold storage, = extract, = chlorine, = control, = acceptance limit.

4. Conclusion

Based on antibacterial testing, *S. polycystum* extract has antibacterial activity against *S. aureus* and *P. aeruginosa* bacteria. *S. polycystum* extract is able to maintain the freshness of tilapia fillets stored in cold temperatures 2 days longer (lasting 6-8 days) than fillets without extract (control) to survive (4-6 days).

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