Analysis Total Plate Counte (TPC) On Fresh Steak Tuna Applications Edible Coating Caulerpa sp During Stored at Chilling Temperature

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Abstract. A study has been conducted to determine the use of Caulerpa sp. Edible coatings on fresh steaks tuna against the presence of microbes during storage at chilling temperatures. In this research, two applied method of edible coating is used, that is dipping and immersion method, with chilling temperature (5°C), storage time (0, 3, 6, 9 days). Each treatment is compared to control (without an edible coating). The results showed that the application of edible coating Caulerpa sp in fresh steaks tuna with soaking and dipping method showed total bacteria increase during storage, but still fulfill the microbiological quality of fresh fish that is maximum total plate 5.0 x 10^5 cfu/g. Total microbes in fresh steaks tuna were soaked with immersion methods and stored up to 9 days: 2.3 x 10^5 cfu/g while total microbial on fresh steaks tuna were dipped by dipping method and stored up to 9 days ie 3.4 x 10^5 cfu/g. So it can be concluded that application method of edible coating Caulerpa sp on fresh steaks tuna soaked better than the method of dipping.

1. Introduction

Along with the increasing population of food needs to be increased, especially fish in the form of fresh. If fresh fish is not handled properly, then there will be a very rapid decline in quality. Biological degradation can occur due to microorganism activity in fish such as bacteria and fungi, while enzymatic damage occurs due to chemical reaction with environment causing rancidity. Changes in freshness quality can take place enzymatically, chemically and bacteriologically with organoleptic decrease [1]. For that fish preservation is necessary to maintain the quality of fish to remain good and can be consumed. Fish that will be consumed or processed must be a fish that is still in fresh condition. If fish quality has decreased, it can cause poisoning in people who consume it [2].

Currently, a new concept is being developed where preservatives as antimicrobial compounds can be made in layers or films on the food surface to maintain longer food durability during storage [3]. Edible coatings or films have been investigated capable of inhibiting moisture, oxygen, aroma, and transport of solutes [4]). Also, edible coating or film is one of the most effective methods of maintaining food quality, as a binder of color, flavor, source of nutrients, and antioxidants. Due to environmental problems, coatings are made from edible biopolymers such as proteins, polysaccharides, and lipids that are usually used as antimicrobials. [4] Edible coating is one layer that is proven to inhibit oxidation. So with edible coating can prevent the occurrence of quality degradation.
and can extend shelf life [5]. Edible coating components are grouped into three, namely hydrocolloids, lipids and composites. Hydrocolloids used in the manufacture of edible coatings are proteins or polysaccharides. Polysaccharides are cellulose and derivatives, starch and derivatives, pectin, seaweed extracts (alginate, carrageenan, agar), gum (arab and gum karaya), xanthan, chitosan and others [6].

The use of edible coating on some food products has been shown to reduce or prevent quality changes and extend the shelf life of the product [3;7;5]. Edible coatings are defined as thin and continuous layers made of edible material, formed overlying food components serving as barriers to mass transfer (moisture, oxygen, light, lipids and solutes) or as an additive food, and improving the handling of a material food. Thus, food products coated with an appropriate edible coating can be protected from damage so that the shelf life can be extended.

The edible layer can be made from polysaccharides, proteins, and fat derivatives that can be used as an efficient shield against vapor and oxygen displacement. One of the natural preservatives of aquatic products that can be used in the manufacture of edible coating is the *Caulerpa* sp marine wine, a type of seaweed from the Chlorophyta group that thrives as an endemic species in the Maluku Kei Islands throughout the year and is known as "lat."

The use of coating (coating) can be used as packaging such as wax, resin, and plastic materials for protective material[8]. The edible coating is an alternative to replacing plastic packaging because it is biodegradable as well as a barrier to control the transfer of water vapor, oxygen uptake, and lipid transfer. Edible coatings can also be used to coat products that serve as a protector of mechanically and safely damaged [9].

Fresh tuna meat steak very quickly experiences quality degradation if not handled quickly because tuna fish has high protein and fat content so that the activity of bacteria and enzymes in meat fish. One handling of tuna steak is by using coating (coating) from seagrass, so it can inhibit the activity of bacteria that can degrade the quality of the fresh tuna steaks.

Based on the above problems the authors are interested to research the existence of total bacteria in fresh tuna steaks application of edible coating *Caulerpa* sp in fresh steaks tuna with soaking and dipping method showed total bacteria increase during storage. The aim of this research is to know the effect of using "lat" on fresh tuna steaks stored at low temperature (5°C) with storage time up to 9 days. The benefits of this study provide information on the total presence of bacteria that can degrade the quality of freshly fresh tuna steaks given "lat" as edible coatings at low-temperature storage as a natural preservative.

2. **Methodology**

2.1. **Materials and tools**

The material used in the manufacture of fresh tuna steaks is fresh tuna fish obtained from the market in Arumbai Ambon City. While the material used for edible coating is from "sea" grapes obtained from Tual waters, Southeast Maluku. Glycerol and wax. The chemicals used are aquades, 0.9% NaCl, PCA. The tools used are knives, trays, duster and scales. While in the microbiological analysis is the scales, measuring glass, erlenmeyer, Petri dish, test tube, autoclave, incubator, counter colonies. This research uses experimental method, that is by direct experiment by doing addition of edible coating from lat as inhibiting regression of fresh tuna fresh steaks kept low temperature with storage time 0, 3, 6, and 9 days.

2.2. **Implementation of Research**

A total of 100 grams of *Caulerpa* sp that has been washed with water to flow clean, blended until smooth. Next add glycerol as much as 1 ml and wax, then stir until homogeneous, then cook the dough on the stove to boil. After a bit cold, apply on the tuna meat ready to be processed and has been cleaned. The application of *Caulerpa* sp. The edible coating on tuna meat is done by immersion and immersion. Tuna meat that has been coating incorporated PE plastic and stored in refrigerator.
2.3. TPC Test (Total Plate Count)
The working principle of TPC analysis is the calculation of the number of bacterial colonies present in the sample with dilution as needed and done duplo. All work is done aseptically to prevent undesirable contamination and duplicate observation can improve accuracy. The number of bacterial colonies that can be calculated is a petri dish that has a bacterial colony between 30-300 colonies. Petri dishes, test tubes and pipettes before use are sterilized in oven at 180°C for 2 hours. The media was sterilized in an autoclave at a temperature of 121°C for 15 minutes at a pressure of 1 atm. After sterilization, to keep the media from freezing the media temperature is maintained at 45-55°C in a water bath. The diluent solution was prepared by dissolving 8.5 grams of NaCl in 1 liter of aquadest which was then sterilized in an autoclave at 121 °C for 15 minutes. The sample of 10 grams was mashed first, then dissolved into a sterile diluent solution that has been contained with a volume reaching 100 ml to obtain dilution $10^{-1}$. The solution is then pelleted 1 ml, then fed into a test tube containing 9 ml of a sterile diluent solution to obtain $10^{-2}$ dilution. And so on until obtained dilution $10^{-5}$. From each tube the dilution reaction is taken by using a pipette of 1 ml then put into a sterilized petri dish. Each dilution is done in duplicate. Then each cup is moved in a circle on the table so that the PCA media evenly. After PCA freezes, the petri dish is incubated in the incubator for 48 hours at 30°C; the petri dish is placed upside down in the incubator.

2.4. Data analysis
Data analysis is done descriptively, data is presented in table form (histogram) and picture (portrait).

3. Result and Discussion

3.1. Making edible coating lat applied to fresh tuna steak
A total of 100 grams of Caulerpa sp that has been washed with water to flow clean, blended until smooth. Next add glycerol as much as 1 ml and wax, then stir until homogeneous, then cook the dough on the stove to boil. After a bit cold, apply to the tuna meat ready to be processed. The application of Caulerpa sp. The edible coating on tuna meat is done by immersion and immersion. Tuna meat that has been coating put into plastic box and PE plastic and stored in refrigerator.

![Figure 1. Edible Coating Caulerpa Applications at Fresh Steak Tuna](image)

2. Total Plate Count At Fresh Steaks Tuna
Foods that contain proteins such as meat and fish are destroyed by bacteria. Food products are rarely sterile and are polluted by some microorganisms because microorganisms are widespread in the natural environment. The growth of microorganisms in or on food can result in unwanted physical and chemical changes, so the food is not feasible for consumption anymore [10]. The most prominent causes of vomiting are microorganisms and various enzymatic and nonenzymatic changes occurring after Pen, slaughter or processing [10]. The calculation of colonies, based on data from each sample is
only calculated dilution with the number of colonies between 30-300. This aims to minimize the probability of error in the calculation. Because the experiment was done twice (Duplo) should then use data from both repetitions by taking the average of the two data, calculated and compared with Indonesian National Standard for fresh tuna quality Results of analysis of total plate count on fresh tuna steaks applied with edible coating lat as shown In table 1.

| Storage Duration (days) | Total Bacteria on fresh tuna steaks without edible coating lat (CFU/g) | Total Bacteria on fresh tuna steaks soaked with edible coating lat (CFU/g) | Total Bacteria on fresh tuna steaks dyed with edible coating lat (CFU/g) |
|-------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|--------------------------------------------------------------------------------|
| 0                       | 2.2 x 10^4                                                                      | 1.8 x 10^4                                                                      | 2.5 x 10^4                                                                      |
| 3                       | 4.6 x 10^5                                                                      | 3.3 x 10^4                                                                      | 3.4 x 10^4                                                                      |
| 6                       | 3.5 x 10^6                                                                      | 3.1 x 10^4                                                                      | 4.1 x 10^4                                                                      |
| 9                       | 2.9 x 10^8                                                                      | 2.3 x 10^5                                                                      | 3.4 x 10^5                                                                      |

Based on the results of TPC analysis in Table 1 above, it can be seen that fresh tuna steaks without edible coating lat have a total value ranging from 2.2 x10^4 to 2.9 x 10^8 cfu /g this indicates that the sample of fresh tuna steaks without edible coating lat is still acceptable Microbiological up to 3 days storage. While fresh tuna steaks applied by edible coating lat with soaking method indicate that as long as the total storage of bacteria has increased, and up to the storage time of 9 days fresh tuna steaks still meet the requirements of microbiology because it still meets the maximum limit of total bacteria in fish maximum 5.0x 10^5 cfu /g. The same is also seen in fresh tuna steaks treated with edible coating lat applications with dyeing methods. Total bacteria increased during storage from 2.4 x 10 ^4 to 3.4 x10^5 cfu /g. The growth of bacterial colonies on fresh tuna steaks as shown in Figure 2.

**Figure 2. Growth of Bacterial Colonies at Fresh Steaks of Tuna**

Bacteria in fresh fish are concentrated on the skin, gills and stomach contents. According [11], at the beginning of storage of total bacteria found in fish is relatively no different, the bacteria contained in fish will increase with the length of storage. This is because the environment can affect the growth of bacteria to the maximum. The treatment of edible coating application on fresh tuna steak with the method of immersion to be the best because it can inhibit the decay activity better than the treatment of edible coating application on fresh tuna steak with the method of dyeing with the old storage for 9 days. Where the value of 2.3 x 10^5 cfu /g which according to SNI 01-2729.1-2006 limit TPC value is 5.0x10^5 colony/g.

According [12], tried the application of crushed seaweed Caulerpa sp. In fresh fish, can suppress the growth of bacteria and is suitable for consumption according to SNI up to 48 hours of storage at room temperature with a log TPC value of 2.7 cfu /g (24 hours) and log 3.7 cfu /g (48 hours). This means Caulerpa has the potential to suppress higher bacterial activity. Furthermore, [13], reported that application of 10% to 30% C. lentilliera sea wine showed the ability to inhibit the growth and
development of bacterial groups of *Coliform* and *Escherichia coli* and according to safety standard of SNI of fresh fish. Fresh fish applied by *C. lentillifera* is feasible to be consumed until the second-day storage at room temperature and third day at cold temperature. *C. lentillifera* sea wine can inhibit the presence of pathogenic bacteria in fresh fish, and a 10% effective enough concentration is applied to inhibit *Escherichia coli* activity and prevent contamination of *Vibrio cholerae* and *Salmonella* sp. During storage of 2 x 24 hours at room temperature and 3 x 24 hours at the rated temperature. Also, several studies of the utilization of natural materials to maintain the quality of fresh fish as reported [14] using ethanol extract of seaweed *Padina* sp. In fresh bloated fish shows the value of TPC proper consumption according to SNI only until the storage of the 6th hour. While [15] shows the value of TPC of fresh tuna with the addition of quercetin is in the range of log 5 cfu/g up to log 8 cfu/g. The addition of 1% quercetin compound from the plant can maintain the safety of fish meat (based on histamine levels) during storage of room temperature up to 20 hours. Furthermore, [10] states that temperature is one of the most important environmental factors that affect life and growth because temperature affects the speed of metabolism and growth.

The mechanism of action of antibacterial compounds, in general, is to damage the structure of the main structure of microbial cells. This shows that the provision of edible coating lat on fresh tuna steak with soaking methods more effectively inhibits microbial growth. Microbial growth in food products is influenced by oxygen displacement during storage. The existence of edible coating then, the transfer of oxygen from the environment to food products can be inhibited so that the growth of aerobic bacteria is also hampered. Edible coating lat on fresh tuna steak can act as an inhibitor of oxygen transfer so that aerobic microbes cannot grow and develop.

### 4. Conclusion

Edible coating lat applied to fresh tuna steaks can give inhibition effect of fresh tuna steak stored at the low temperature (5°C) effect on total bacteria during storage at cold temperature.

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