Chitosan from snapper fish scale waste (*Lutjanus* spp.) for edible coating

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Abstract. Indonesia is known for its rich fisheries sector. The portion of fish that can be consumed is only 40-50% of the total, the rest is disposed of as waste. Chitosan can be found in fish scales. This study aims to determine the effect of chitosan edible coating from scale waste in extending the shelf life of grapes and the level of consumer acceptance of chitosan edible coating. Chitosan was extracted through deproteination, demineralization, and deacetylation process. Chitosan solutions were made with various concentrations. Based on the results, the chitosan obtained had a yield of 36.32%, water content 3.86%, and ash content 89.54%. In the antibacterial activity test, the inhibition zone formed by chitosan was smaller than 0% chitosan. In the calculation of the De Garmo test obtained the concentration of 0.5% was the best concentration of chitosan. Based on the results of the shelf life estimation test, chitosan coating treatment still cannot replace paraffin wax in extending the shelf life of grapes. In the organoleptic results between samples with coating compared to samples without coating, the results show that the provision of chitosan coating can be accepted by consumers without changing the organoleptic characteristics of the grapes.

1. Introduction

Indonesia as a maritime country has a sea area of 5.8 million km² with a coastline of 81,800 km. With these water areas, it is estimated that Indonesia has a potential in the marine fisheries sector of 6.4 million tons per year. 80% or 5.12 million tons per year of this potential is the amount of fish caught [1]. One type of fish that is much favored by the community is red snapper (*Lutjanus* spp.). The edible portion of fish meat (edible portion) is only 40-50% of the total [2]. Fish scales are one of the types of waste from fish that have not been used optimally. Fish scales can be used as a source of collagen, gelatin, and chitosan.

Chitin is a bio-polymer substance that is abundant in nature with non-toxic and biodegradable properties. Chitin is a renewable and most abundant natural resource after cellulose [3]. Chitin is commonly found in the outer layers of crustaceans, such as marine invertebrates, insects, fungi, and yeasts. In addition to crustaceans, chitin can also be found in fish scales.

Chitosan is a derivative of chitin which is formed from the extraction of the outer layer of crustaceans and fish scales through the process of removing the acetyl group which leaves a free amine group. Chitosan in the food sector can be used as an ingredient to increase the durability of various food products because it has anti-bacterial activity [4] and can form a good film layer [5]. Chitosan can be applied to fruit as an edible coating by dipping, soaking, or spraying [6].
The biggest challenge in storing fruits and vegetables is their short shelf life. This is because after harvesting fruits and vegetables will continue to undergo metabolism such as respiration and transpiration as well as infection from microbial spoilage. One way to maintain the freshness of these fruits and vegetables is preservation. The method of preserving fruit and vegetables that are commonly used is coating with wax. Eating too many fruits or vegetables coated with synthetic wax can endanger health [7]. The application of natural edible coatings is a promising alternative to maintain the quality and extend the shelf life of fruits and vegetables [8]. The advantages of using natural edible coatings are that they are more environmentally friendly, have antibacterial activity, are non-toxic, and are biodegradable.

Due to the abundance of fish waste and the potential for its use, it is necessary to utilize marine fish waste as a raw material for chitosan which will be applied as an edible coating to extend the shelf life of fruit products and reduce the impact of waste pollution and the application of “zero waste” in fish processing. This study aims to determine the effect of chitosan edible coating from fish waste in extending the shelf life of green grapes as an alternative to synthetic wax chemical preservatives and to determine the level of consumer acceptance of chitosan edible coating from fish waste as an alternative to synthetic wax chemical preservatives.

2. Materials and Methods

2.1. Materials

The materials used in this study were fish waste (red snapper scales), 4.2% NaOH, 1.04 N HCl, 80% NaOH, filter paper, universal indicator pH 0-14, I₂-KI, concentrated H₂SO₄, CH₃COOH 1% (food grade), distilled water, rubbing alcohol, 1000 L micro tip, 100 L micro tip, *Escherichia coli* culture, *Staphylococcus aureus* culture, nutrient agar (NA), 0.85% NaCl, 70% alcohol, paraffin wax, and green grapes with the same level of maturity and weight.

The tools used are stove, pan, oven, blender, analytical scale, 70 mesh sieve, hotplate stirrer, magnetic bar, stainless spatula, digital thermometer, glassware, centrifuge, falcon tube, aluminum foil, spectrophotometer, plastic cuvette, cylinder cup, petri dish, bunsen, tweezers, ose needle, vortex, test tube rack, porcelain cup, furnace, desiccator, calipers, 10-100 L micropipette, 100-1000 L micropipette, and autoclave.

2.2. Methods

The variables in this study were variations in the concentration of chitosan (0.5%, 1%, 1.5%, and 2%). The parameters measured in chitosan were physical characteristics including yield, chemical characteristics including water content and ash content, inhibition zone diameter, weight loss test, color, and texture changes. Each parameter measured was repeated three times. In addition, the shelf life estimation of grapes that have been coated with chitosan and organoleptic tests in the form of hedonic quality tests and description tests were also carried out on 41 untrained panelists.

The manufacture of chitosan begins with the manufacture of fish scale flour. Fish scale flour was then isolated from the chitosan by deproteination, demineralization, and deacetylation processes. The deproteination process was carried out using a 4.2% NaOH solution with a ratio of 1:6 (w/v) at 60°C for 5 hours. The deproteination results were then washed with distilled water until neutral and dried in an oven at 50°C for 12 hours. Then the deproteinized chitin was demineralized using 1.04 N HCl solution with a ratio of 1:6 (w/v) at room temperature for 6 hours. The demineralized results were then washed with distilled water until neutral and dried in an oven at 50°C for 12 hours. After the demineralization process, chitin was deacetylated to produce chitosan using 80% NaOH solution in a ratio of 1:3 (w/v) at 110°C for 4 hours. The result of deacetylation was then washed with distilled water until neutral and dried in an oven at 50°C for 12 hours. The extracted chitosan was tested for its physical and chemical characteristics, namely yield, moisture content, and ash content. Then, the antibacterial activity was tested against *Escherichia coli* and *Staphylococcus aureus*. To make a solution of chitosan with various concentrations, the chitosan powder was dissolved in 1% acetic acid with different w/v ratios (0.5%,
1%, 1.5%, and 2%) at 30°C for 15 minutes. Variations in chitosan concentration were tested using the De Garmo effectiveness index method to determine the best concentration of chitosan. The best concentration of chitosan will be used for estimating the shelf life of grapes and organoleptic tests. This research was conducted in the form of experimental research conducted in a laboratory with a completely randomized design. Data analysis was carried out using SPSS software, for data analysis of shelf life estimation using the ASLT Arrhenius model method was carried out by simple linear regression with Microsoft Excel software and for the selection of the best chitosan concentration was carried out using the De Garmo method. All data were tested by the Saphiro-Wilk normality test and homogeneity test by Levene's variance test. The data on the diameter of the inhibition zone and the determination of the best chitosan concentration were processed with one-way ANOVA and if the statistical results showed a significant difference, which was indicated by a Pvalue <0.05, then it was continued with a significance test with multiple comparison analysis using the Tukey HSD method with a confidence level of =0.05. Physical and chemical characteristics data were processed using a T-test.

3. Results and Discussion

Table 1. Chitosan Physicochemical Characteristic Test

| Parameter      | Chitosan     |
|----------------|--------------|
| Yield (%)      | 36.32 ± 0.38 |
| Water Content (%) | 3.86 ± 0.3   |
| Ash Content (%) | 89.54 ± 0.49 |

The physicochemical characteristics test of chitosan included the yield test, water content, and ash content. Characteristic tests were carried out to determine the efficiency of the chitosan isolation process from fish scales. The characteristic test was used to determine the quality and quality of chitosan.

Table 2. Diameter of the clear zone chitosan antibacterial activity against *Escherichia coli* and *Staphylococcus aureus*

| Bacteria Type | Clear Zone Diameter (mm) | 0% (Control -) | 0.5% | 1%   | 1.5%   | 2%   |
|---------------|--------------------------|----------------|------|------|--------|------|
| *Escherichia coli* |                          | 4.01 ± 1.31    | 3.35 ± 0.23 | 2.11 ± 0.65 | 2.09 ± 0.97 | 2.52 ± 0.78 |
| *Staphylococcus aureus* |                        | 5.97 ± 1.04    | 4.59 ± 0.83 | 2.69 ± 1.15 | 2.32 ± 0.91 | 2.37 ± 0.7 |

Based on statistical tests, all the data from the anti-bacterial activity test results had a normal and homogeneous data distribution, which was indicated by Pvalue>0.05 in the normality and homogeneity tests. Based on the results of the ANOVA statistical test, it was shown that the variation in the concentration of chitosan was significantly different from the diameter of the clear zone of *Staphylococcus aureus* bacteria, which was indicated by a Pvalue <0.05. Then continued with the multiple comparison test using Tukey's method, showing that the antibacterial activity of 0% and 0.5% chitosan concentrations were not significantly different. Likewise, the antibacterial activity of 0.5% chitosan concentration and the anti-bacterial activity of 1%, 1.5% and 2% chitosan concentrations. However, the antibacterial activity of 0% chitosan concentration was significantly different from the antibacterial activity of 1%, 1.5%, and 2% chitosan concentrations.
**Table 3. Test Results Determination of the Best Treatment**

| Chitosan Concentration (%) | Weight Loss Parameter | Color Parameter | Texture Parameter | Antibacterial Activity Parameter | Amount | Rank |
|---------------------------|-----------------------|-----------------|-------------------|----------------------------------|--------|------|
| 0%                        | 0.157895              | 0               | 0.421053          | 0.22449                          | 0.803437 | 2    |
| 0.5%                      | 0.210526              | 0.368421        | 0.315789          | 0.168367                         | 1.063104 | 1    |
| 1%                        | 0.052631579           | 0.184210526     | 0                 | 0.056122449                      | 0.292964554 | 5    |
| 1.5%                      | 0.105263              | 0.092105        | 0.210526          | 0                                | 0.407895 | 4    |
| 2%                        | 0                     | 0.276316        | 0.105263          | 0.112245                         | 0.493824 | 3    |

Based on the results of data analysis using the De Garmo method, it was found that the best chitosan concentration was 0.5% with a score of 1.063104. The variation of chitosan concentration with the lowest ranking was chitosan with a concentration of 1% with a total score of 0.292964554.

**Table 4. Results of Calculation of Fruit Shelf Life at Various Storage Temperatures Based on Color Difference Parameters**

| Treatment     | Temperature (°C) | k Value            | Shelf Life (days) |
|---------------|------------------|--------------------|-------------------|
| Paraffin Wax  | 4.16 ± 1.42      | 0.067838977        | 23                |
|               | 12.94 ± 1.22     | 0.103271194        | 19                |
|               | 23.94 ± 0.29     | 0.168811012        | 17                |
|               | 4.16 ± 1.42      | 0.118981678        | 16                |
| Chitosan 0.5% | 12.94 ± 1.22     | 0.18234246         | 17                |
|               | 23.94 ± 0.29     | 0.30040688         | 18                |

Based on the calculation results, it can be seen in the paraffin wax treatment that the higher the storage temperature, the shorter the shelf life of the grapes. Meanwhile, in the treatment with 0.5% chitosan, the shelf life of the fruit got longer along with the higher the storage temperature. The measured shelf life of the coating treatment with 0.5% chitosan was shorter than the shelf life of grapes with coating treatment with paraffin wax.

**Table 5. Organoleptic Test Results (Hedonic Quality)**

| Organoleptic properties | Preferred Test Value |
|-------------------------|----------------------|
| Texture                 | 4.29 ± 0.78          |
| Aroma                   | 3.71 ± 0.87          |
| Taste                   | 4.39 ± 0.74          |
| Color                   | 4.15 ± 0.69          |
| Overall sighting        | 4.2 ± 0.64           |
To determine whether the test sample was organoleptically acceptable, an organoleptic test was carried out in the form of a hedonic and descriptive quality test involving 41 untrained panelists. Parameters tested in hedonic quality testing include texture, aroma, taste, color, and overall appearance of the fruit.

Table 6. Organoleptic Test Results (Descriptive)

| Attributes         | Grape With Chitosan Coating 0.5% | Grape Without Coating |
|--------------------|----------------------------------|-----------------------|
| Fruit brightness   | 3.76 ± 0.73                      | 3.85 ± 0.73           |
| Grape aroma        | 3.51 ± 0.93                      | 3.8 ± 0.78            |
| Fruit freshness    | 4 ± 0.63                         | 4.1 ± 0.62            |
| Fruit flavor       | 3.8 ± 0.78                       | 3.73 ± 0.78           |
| Texture (Firmness) | 3.68 ± 0.79                      | 3.68 ± 0.72           |

Based on the results of statistical tests using the Kruskal Wallis test, it was found that there was no difference between the two samples on all attributes. Panelists' assessment of the parameters of fruit brightness, aroma, fruit freshness, taste, and texture in the descriptive test did not differ much between grapes with coating and grapes without coating.

3.1. Physicochemical Characteristics of Chitosan

Yield is one of the important parameters in the manufacture of chitosan where the yield value is used to determine the efficiency and effectiveness of the chitosan extraction process [9]. The yield of chitosan produced in this study was 36.318%. Based on the results of chitosan obtained, the chitosan extraction process has been efficient where the yield obtained is in accordance with the literature. In a study conducted by [10], the yield of chitosan obtained from red snapper scales ranged from 11.49% to 25.19%. The amount of chitosan yield produced is influenced by the type of fish used, the concentration of NaOH used and the immersion setting in the chitin deacetylation process into chitosan [11].

Water content is one of the important parameters in determining the quality of chitosan. The physicochemical properties of chitosan are known to affect solubility, viscosity, and other characteristics [12]. Based on the research results, the water content of chitosan is 3.86%. The water content of the chitosan obtained is good because the water content contained is below 10% which is in accordance with SNI that the maximum water content of chitosan is 12%. According to [13], chitosan used as a biopreservative must have a water content below 10%. It has also been established that the quality standard by Protan Biopolymer for chitosan water content is 10% [14,15].

Ash content is a parameter to determine the minerals contained in a material. Ash content can also indicate the success rate of the demineralization process carried out. The lower the ash content produced, the higher the quality and level of purity of chitosan [9]. In this study, the ash content of chitosan was 89.54%. This indicates that the mineral content contained in chitosan is still very high. The high mineral content in chitosan indicates that the demineralization process is still incomplete [9]. Several factors that affect the demineralization process are the concentration of HCl used, the temperature and time of demineralization, stirring during the demineralization process, and the washing process [16].

3.2. Antibacterial Activity

Chitosan has the advantage of having high antimicrobial activity with a broad spectrum of activity and low toxicity to mammals [5]. Based on the results, it was found that the chitosan solution could produce
an inhibitory zone against *Escherichia coli* and *Staphylococcus aureus* but decreased along with the increase in the concentration of chitosan. In a chitosan solution with a concentration of 0% chitosan, an inhibitory power with the largest clear zone diameter arises. This happens because the presence of acetic acid in the solution was as a solvent. The factor that causes the diameter of the inhibition to decrease is the quality of the chitosan obtained is not good. The poor quality of chitosan causes chitosan to be insoluble in the acetic acid solvent so that the chitosan settles to the surface of the media. The higher the solubility of chitosan indicates that the quality of the chitosan produced is getting better [9]. Several things that can affect the quality of chitosan are the conditions of the chitosan extraction (demineralization and deproteination processes), the temperature and time used during the deacetylation process, the concentration of the solvent used, and the size of the material used [16].

### 3.3. Determination of The Best Chitosan Concentration

In determining the best chitosan concentration, several parameters were measured on the coated grapes with variations in the concentration of chitosan used were 0.5% (w/v), 1% (w/v), 1.5% (w/v), 2% (w/v) and without coating. Parameters measured were physical characteristics which included weight loss, color change, and texture of grapes with a storage period of 7 days at room temperature (± 26°C). To determine the best chitosan concentration treatment that will be used for further testing, the determination of the shelf life of grapes is carried out by combining the data on the results of the antibacterial activity with data from physical characteristics and tested using the De Garmo effectiveness index test method. Determination of the best treatment from the results of the analysis using the De Garmo method was determined based on the highest total value and the result was that the highest total was found in chitosan with a concentration of 0.5%. Furthermore, chitosan with a concentration of 0.5% will be used as a coating on grapes for shelf-life testing.

### 3.4. Estimating The Shelf Life of Grapes

Shelf life is the time span of product storage until the product is rejected by consumers. The shelf life of grapes was determined using the Accelerated Shelf-Life Testing (ASLT) method using the Arrhenius approach which was simulated at three storage temperature conditions, namely 4°C, 13°C and 24°C. In this study, shelf life was determined from the total color difference parameter (ΔE). The color of the grapes is an indicator of the quality of the fruit. The value of the total color difference can be determined by measuring the color change in the grapes during each treatment during storage.

Based on the results, it was found that the higher the storage temperature in the paraffin wax treatment, the shorter the shelf life of the fruit. This shows that an increase in temperature can cause the rate of chemical reactions to accelerate so that the product quality declines more quickly [17]. Meanwhile, in 0.5% chitosan treatment there was an increase in the shelf life of grapes along with the increase in storage temperature. This is thought to be due to the color uniformity on each side of the grapes so that the measured color will be slightly different. The shelf life of grapes on coating treatment with 0.5% chitosan at all storage temperature conditions is lower than the shelf life of grapes on coating treatment with paraffin wax. Grapes treated with paraffin wax coating stored at 4°C, 13°C and 24°C had a shelf life of 23 days, 19 days, and 17 days. Meanwhile, grapes with 0.5% chitosan coating treatment stored at 4°C, 13°C, and 24°C had a shelf life of 16 days, 17 days, and 18 days. This shows that coating treatment with 0.5% chitosan still cannot replace synthetic coatings in extending the shelf life of grapes. Coating treatment with 0.5% chitosan has not been able to extend the shelf life of the fruit, it can be caused by the inappropriate formulation of the edible coating so that the surface of the fruit cannot be protected properly [18].
3.5. Consumer Acceptance Rate Analysis

Based on the test results for determining the best concentration of chitosan using the De Garmo effectiveness index method, it was found that the chitosan treatment with a concentration of 0.5% was chosen as the best treatment. Chitosan with a concentration of 0.5% will be used as a coating in the organoleptic test. On the attributes of texture, taste, color, and overall appearance in the hedonic quality test, the panelists gave an average value of 4.3 for texture, 4.4 for taste, 4.1 for color, and 4.2 for overall appearance. This response can be interpreted that the panelists like the sample given in terms of texture, taste, color, and overall appearance. On the aroma attribute, the panelists gave a score with an average of 3.71, where this response was interpreted that the panelists rated neutral as close to liking the aroma attribute.

After the hedonic quality test, further tests were carried out with descriptive tests. Based on the results of Kruskal Wallis' analysis, it was found that there was no difference between samples on all attributes. In the fruit brightness parameter, the sample of grapes with coating got an average value of 3.76 and samples of grapes without coating got an average value of 3.85. This response can be interpreted that the panelists judged that the grapes samples with coating and without coating had similar brightness levels. On the attribute of fruit aroma, respondents gave a neutral assessment for both samples with an average value of 3.51 for samples of grapes with coating and 3.8 for samples of grapes without coating. For the freshness attribute of the fruit, the panelists judged that the grapes with and without coating looked fresh. For the taste attribute, the panelists considered that the grapes with coating and without coating had a fairly sweet taste with an average value of 3.8 for grapes with coating and 3.73 for grapes without coating. In terms of texture parameters, both the coated and uncoated grapes were judged that they had adequate texture. Based on the panelists assessment results, it can be concluded that the grapes with the coating do not have much difference from the grapes without the coating.

4. Conclusions

From the research that has been done, it can be concluded that the provision of natural edible coatings of chitosan still cannot replace paraffin wax in extending the shelf life of grapes but can extend the shelf life of grapes when compared to grapes without coating. In addition, the provision of natural edible coating of fish waste chitosan can be accepted by consumers as a substitute for paraffin wax without changing the organoleptic characteristics of grapes. Suggestions that can be given for further research are the need to optimize chitosan extraction, chitosan purification process, identification test of chitosan deacetylation degrees, and optimization of formulations for chitosan edible coatings.

5. References

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