

Utilization Of Chitosan As A Natural Preservative Against Catfish

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Abstract.

Shrimp in Indonesia are generally exported abroad after removing the head, tail, and skin. One alternative to the use of shrimp shell waste that has high economic value is processing shrimp shells into chitosan. The purpose of this study was to determine the shelf life of catfish using chitosan and to determine the optimal concentration of chitosan in catfish preservation. Chitosan applied to catfish showed that in the addition of a 0% chitosan solution, it had a shelf life of up to day 2 for 48 hours for a 0.5 solution; 1; 1.5 and 2% shelf life up to day 5 or for 120 hours. The most optimal concentration of chitosan for use in catfish is a 2% chitosan solution, and the total plate number (ALT) test meets the requirements of the SNI standard so that it can still be consumed.

Keywords: Chitosan, natural preservative, catfish

I. INTRODUCTION

Catfish (*Clarias* sp.) is one type of freshwater fish that belongs to the order Siluriformes and is classified as a true bony fish. The catfish is characterized by its smooth and elongated body as well as the presence of tentacles protruding from the area around its mouth. The scientific name of catfish is (*Clarias* sp.), which comes from the Greek "chlaros", meaning "strong and agile". In English, catfish are called by several names, such as: catfish, mudfish, and walking catfish (Justisia, 2016). Generally, fish are sold in the market in a fresh state, but when the catch is abundant, the fishermen do preservation so as not to rot. Generally, fish are easily damaged (rotten). Stored in normal conditions (room temperature), the average durability is 1–3 days. After more than that limit, the taste becomes bad and the container gradually rots, so it is not suitable for consumption anymore. For this reason, it is necessary to make efforts to preserve it with natural ingredients that are not harmful to health. The widespread use of food additives as preservatives that are not permitted to be used in foods, such as formalin and borax, is harmful to health (Mahatmanti et al., 2011) and has encouraged many parties to seek healthier alternative preservatives. One of the natural ingredients that can be expected as a potential alternative preservative is chitosan (Kusumaningjati, 2009). Chitosan is made from shrimp shells, crabs, snail shells, and so on. Several studies on the utilization of snail shells into chitosan obtained a degree of deacetylation of 65% (Ridwanto et al., 2016); on tiger shrimp shells the percentage of deacetylation was 60% (Saragih et al., 2022); on crab shells the degree of acetylation was 82.84% (Ridwanto et al., 2022); and 82.73% for the shells of the coral crab (Ridwanto et al., 2021). The content of chitin in shrimp shells is less than crab shells, but shrimp shells are easier to obtain and are available in large quantities. Currently, the processing of shrimp shell waste in Indonesia is still not optimal and is limited to a mixture of animal feed. Whereas shrimp shell waste can be a product of high economic value, namely by managing shrimp shell waste into chitin and chitosan. Chitosan applications are found in various fields of modern industry, such as pharmaceuticals, biochemistry, biotechnology, the food industry, and others (Kaban, 2009).

Chitosan has the potential to be used as an antimicrobial agent because it contains lysozyme enzymes and polysaccharide amino groups that can inhibit microbial growth. The ability to suppress bacterial growth is due to the fact that chitosan has a positively charged polycation that is able to inhibit the growth of bacteria and molds (Wardaniati, 2009). Antibacterial is a substance that inhibits the growth of bacteria and is used specifically to treat infections. Preservatives are compounds that are able to inhibit and stop the processes of fermentation, acidification, or other forms of damage, or materials that can provide food protection from spoilage (Kusumaningjati, 2009). Several researchers have shown that coating with

chitosan can extend shelf life and freshness and protect the product from damage. Chitosan at a concentration of 1.5% was reported to give the best results in maintaining fruit quality. Treatment was also carried out on salak fruit with a concentration of 0.5% at a temperature of 15°C, which was proven to be able to inhibit damage to salak fruit during storage, both chemical and physical damage (Styasih, 1997). According to Suseno (2006), the use of chitosan with a concentration of 1.5% in dried salted mackerel can extend the shelf life by up to 3 months. According to Sedjati (2006), the concentration of chitosan on the water content of salted anchovy (*Stolephorus heterolobus*) with a storage time of 0 weeks was not different from that for 2 weeks, but was significantly different from storage for 4, 6, and 8 weeks. The purpose of this study was to determine the shelf life of catfish using chitosan (commercial chitosan from shrimp shells) as a natural preservative and to test bacterial colonies using the total plate number (ALT) method to see its potential as a preservative.

II. METHODS

2.1 Tools

The tools used in this study were: glassware, stirring rod, pipette mat (10, 5, and 1 ml), oven, scales, universal pH indicator, Bunsen lamp, Quebec coloni counter, spray bottle, aluminum foil, hot plate, petri dish, mortar, stamper, water bath, wool thread, plastic pot, spatula, parchment paper, autoclave, incubator (memmert), ose wire, and test tube.

2.2 Materials

The materials used in this study were: commercial shrimp chitosan, distilled water, 1% acetic acid, 70% alcohol, spiritus, NaCl 0.9%, Nutrient Agar (NA), Sodium Benzoate, catfish.

2.3 Preparation of Chitosan Solution

To obtain a 0.5% chitosan solution, 0.5 grams of commercial chitosan powder from shrimp shells was used. Then dissolved in 1% acetic acid as much as 15 ml to form a suspended solution. Then the solution is then diluted with 100 ml of distilled water. Then a solution of chitosan was produced for a concentration of 0.5%. Likewise, for the manufacture of 1% chitosan solution, 1 gram of chitosan powder is needed, for 1.5% chitosan solution, 1.5 grams of chitosan powder is required, and 2 grams of 2% chitosan powder is required.

2.4 Application of Chitosan Solution to Catfish

The results of the chitosan solution of each concentration are put in a glass beaker that has been given a sticker. Code A for beakers of 0% chitosan solution (blank), code B for beakers of 0.5% chitosan solution, code C for beakers of 1% chitosan solution, code D for beakers of 1.5% chitosan solution, and code E for beakers of 2% chitosan solution, and code F for sodium benzoate (as a standard for comparison). Then each beaker was filled with catfish and chitosan solution for each concentration. The treatment of each concentration was carried out twice (in the refrigerator and outside). Then let the catfish sit in the soak for 1 hour. After 1 hour, the catfish were tested by the total plate number (ALT) method against bacteria for two days, and then we observed the changes that occurred in the catfish. good texture, smell, and color, as well as calculated storage time. From each treatment, it was seen that catfish with what concentration had a longer storage time without any changes in texture, smell, or color. Then tabulate the results of the data obtained and analyze them descriptively.

2.5 Sample dilution

The dilution of the sample was carried out using 0.9% sodium chloride, which had been prepared in a sterile condition and was used to test the total bacterial plate number. This dilution is carried out with several dilutions, namely 10^{-1} ; 10^{-2} ; 10^{-3} ; and 10^{-4} .

2.6 Determination of Bacterial Count (Total Plate Number)

According to standard operating procedures for microbiological testing (Depkes RI, 1989), each sample dilution uses sterile 0.9% sodium chloride. 1 ml of each pipette was put into a sterile petri dish that had been marked according to the level of dilution. In each petri dish, 14–20 ml of nutrient agar (NA) at 45–50°C were poured, shaken, and immediately rotated in such a way that it was evenly distributed. After the

media has solidified (frozen), it is incubated in an incubator at 37°C for 24 hours in reverse. After the incubation period is complete, the number of colonies grown is calculated using a Quebec colony counter.

2.7 Organoleptic Test

To find out the results of the experiment, it is necessary to conduct an assessment of the community through organoleptic tests. An organoleptic test is an assessment that uses the senses. The type of organoleptic test used is a preference or hedonic test, which states whether you like or dislike a product.

III. RESULTS AND DISCUSSION

The results of a study conducted on the use of chitosan from shrimp shells (*Litopenaeus vannamei*) as a natural preservative for catfish were: 5 treatments of soaking catfish in chitosan solution with a concentration of 0% as control, 0.5; 1; 1.5; and 2%, and sodium benzoate (for comparison). The catfish sample required for each treatment in the study was 1 piece of catfish. Then the catfish samples were preserved by soaking in the chitosan solution with concentrations of 0% (as control), 0.5, 1, 1.5, and 2%, respectively. Soaking catfish for 1 hour aims to maximize the absorption of chitosan into catfish. This refers to research conducted by Setyaningsih that suggests the shelf life of tofu will be optimal when soaked for 60 minutes. One hour later, the catfish was taken and stored. Then the catfish are stored at room temperature and in the refrigerator. Then, they observed changes that occur in catfish in terms of texture, smell, and color and calculated the storage time of catfish. From each treatment, catfish were seen with the concentration of how long the storage time was without any changes in texture, smell, or color.

Catfish added with chitosan 0; 0.5; 1; 1.5; and 2%, given to students after the second day of adding catfish and before catfish was added at a concentration of 0% left to rot, the aim was for panelists to be able to distinguish color, taste, aroma, and texture among some of the added catfish. chitosan solution of these concentrations. The organoleptic test was carried out at 10.00–13.00 on 20 respondents with a rating of 1-3 and analyzed using descriptive analysis. The panelists are 20 students who are still actively studying. When asked for a response/assessment, visually the panelists were not in pain, had no physical defects in the organs used for evaluation, and were in a stable emotional state. Based on observations of the total number of plates in bacteria with 3 dilutions using sterile 0.9% sodium chloride and nutrient agar (NA) media on catfish from various variations of chitosan solution, it can be seen in the table below.

Table 1. Number of plates total days 1 and 2

| Day | Concentration | Dilution | Count of Colonies |
|-------------|---------------|------------------|-------------------|
| Day 1 and 2 | 0,5% outside | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 0,5% ice | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 1% outside | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 1% ice | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 1,5% outside | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 1,5% ice | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 2% outside | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |
| | 2% ice | 10 ⁻² | 0 |
| | | 10 ⁻⁴ | 0 |
| | | 10 ⁻⁶ | 0 |

Table 2. Number of plates total days 3 and 4

| Day | Concentration | Dilution | Count of Colonies |
|-------------|---------------|-----------|-------------------|
| Day 3 and 4 | 0,5% outside | 10^{-2} | 48 |
| | | 10^{-4} | 56 |
| | | 10^{-6} | 67 |
| | 0,5% ice | 10^{-2} | 66 |
| | | 10^{-4} | 78 |
| | | 10^{-6} | 90 |
| | 1% outside | 10^{-2} | 48 |
| | | 10^{-4} | 67 |
| | | 10^{-6} | 78 |
| | 1% ice | 10^{-2} | 65 |
| | | 10^{-4} | 87 |
| | | 10^{-6} | 98 |
| | 1,5% outside | 10^{-2} | 61 |
| | | 10^{-4} | 77 |
| | | 10^{-6} | 84 |
| | 1,5% ice | 10^{-2} | 66 |
| | | 10^{-4} | 73 |
| | | 10^{-6} | 89 |
| | 2% outside | 10^{-2} | 45 |
| | | 10^{-4} | 56 |
| | | 10^{-6} | 70 |
| | 2% ice | 10^{-2} | 51 |
| | | 10^{-4} | 65 |
| | | 10^{-6} | 73 |

Table 3. Number of plates total days 5 and 6

| Day | Concentration | Dilution | Count of Colonies |
|-------------|---------------|-----------|-------------------|
| Day 5 and 6 | 0,5% outside | 10^{-2} | 120 |
| | | 10^{-4} | 170 |
| | | 10^{-6} | 200 |
| | 0,5% ice | 10^{-2} | 108 |
| | | 10^{-4} | 119 |
| | | 10^{-6} | 135 |
| | 1% outside | 10^{-2} | 102 |
| | | 10^{-4} | 156 |
| | | 10^{-6} | 178 |
| | 1% ice | 10^{-2} | 98 |
| | | 10^{-4} | 112 |
| | | 10^{-6} | 122 |
| | 1,5% outside | 10^{-2} | 136 |
| | | 10^{-4} | 162 |
| | | 10^{-6} | 183 |
| | 1,5% ice | 10^{-2} | 99 |
| | | 10^{-4} | 117 |
| | | 10^{-6} | 132 |
| | 2% outside | 10^{-2} | 97 |
| | | 10^{-4} | 104 |
| | | 10^{-6} | 93 |
| | 2% ice | 10^{-2} | 82 |
| | | 10^{-4} | 90 |
| | | 10^{-6} | 85 |

Table 7. Number of plates total days 7 and 8

| Day | Concentration | Dilution | Count of Colonies |
|-------------|---------------|-----------|-------------------|
| Day 7 and 8 | 0,5% outside | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 0,5% ice | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 1% outside | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 1% ice | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 1,5% outside | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 1,5% ice | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 2% outside | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |
| | 2% ice | 10^{-2} | ∞ |
| | | 10^{-4} | ∞ |
| | | 10^{-6} | ∞ |

Description: ∞ = Infinity (>300 colonies)

In this study, researchers used chitosan from shrimp shells (*Latopvnamei*) as a natural preservative for catfish. Generally, catfish are easily damaged or rotten. Stored under normal conditions (room temperature), the average durability is only 1-2 days. After more than that limit, the taste becomes bad and then gradually rots, so that it is not suitable for consumption anymore. Based on the total plate number test table that has been carried out, it is clear that the length of storage time for catfish with the addition of 0% chitosan solution (without chitosan) has a shelf life of up to 2 for 48 hours, while catfish soaked in 0.5 chitosan solution; 1; 1.5 and 2% of the storage time of catfish, which can be stored up to the 5th day or for 120 hours, but the number of bacteria does not exceed the required limit. The number of bacteria based on fish quality standards is not more than 300 colonies. The optimal storage time for catfish is with the addition of a 2% chitosan solution because it has the best bacterial inhibition. It can be seen from the table above that there are only 93 colonies on the outside, while in the refrigerator there are 85 colonies on the 106th day. This is more optimal than sodium benzoate with the permissible level of 1 g/1 kg of fish.

On the third day, the fish with the addition of benzoate has exceeded the allowable threshold. In general, food spoilage is caused by the growth of microorganisms, especially bacteria and fungi. Like other living things, microorganisms need nutrients such as carbohydrates, proteins, fats, and minerals. Microorganisms convert nutrients into energy that is used for the growth of microorganisms (Irianto, 2006). The concentration of chitosan required for food preservation varies depending on the type of food (Suhardjo, 1992). In this study, the optimal concentration of chitosan for use in catfish is a concentration of 2% with a long shelf life of up to day 5 or 120 hours, where the texture of the fish is good, the fish smells good, and the color of the fish is good. The ability to suppress bacterial growth is due to the fact that chitosan has a positively charged polycation that is able to inhibit the growth of bacteria and molds. One of the mechanisms that may occur in food preservation is that chitosan molecules have the ability to interact with compounds on the surface of bacterial cells and then be adsorbed to form a layer that inhibits cell transport channels so that cells experience a lack of substances to develop and result in cell death (Sugita et al., 2009).

IV. CONCLUSION

The storage time of catfish after immersion in 2% chitosan solution can last up to day 5, when the physical characteristics of catfish show good texture, smell, color, and taste. Chitosan from shrimp shells can increase the shelf life of catfish based on the total plate number (ALT) test for bacteria. All concentrations of chitosan meet the requirements of SNI, but the optimal concentration of chitosan for use in catfish is a concentration of 2% with a long shelf life of up to 5 days. ice for 120 hours, and the number of colonies was 93 at 106 outside, while ice was 85 at 106 in the refrigerator.

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