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THE EFFECT OF CHITOSAN-BASED EDIBLE COATING ON THE SHELF LIFE OF STEAMED SHRIMP BASED ON THE NUMBER OF BACTERIA

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Antibacterial, chitosan, low temperature, microbe, steamed shrimp

ABSTRACT

Steamed shrimp are susceptible to degradation such as color changes and microorganism activity. Therefore, to minimize the deterioration of quality in steamed shrimp, chitosan is added as edible coating because chitosan has antibacterial properties. This study aims to inhibit the deterioration of steamed shrimp with chitosan as edible coating during the storage period. The method used in this research is experimental method. Steamed shrimp were immersed with 0%, 1.5%, 2%, 2.5%, and 3% chitosan addition for 3 minutes, packed with Styrofoam and plastic wrap at low temperature (5-10°C). The observations were performed on day-1, 3, 4, 5, 7, 8, 9, 10, 11, 12 and day-13. The parameter observed in this study were total plate count (TPC). The result concluded that 2% of chitosan concentration is the optimal concentration for steamed shrimp store at low temperature at day 12, with total microbial count $3.6x10^5$ cfu/g

1. INTRODUCTION

Indonesia has about 900,000 hectares of water territory with sources of marine organisms that can be utilized [1]. Shrimp is one of the marine products and an important component of shrimp fisheries in Indonesia. Shrimp exports to developed countries in 2017 reached US $ 924.1 million [2]. Shrimp has high protein ranging from 20.44% - 22.46% [3], a delicious taste of meat and is very popular with the community. One way to process shrimp that can be done is steaming. Steaming prior to freezing or drying is mainly done to inactivate enzymes that can cause color fading, changes in taste and the appearance of unpleasant odors during storage [4]. Biological quality degradation can also occur due to the activity of microorganisms present in these organisms, for example caused by bacteria or fungi.

According to [5] stated that chitosan as an edible coating can function as an antimicrobial which is commonly used in the preservation process. Edible coating is a food coating material that can be eaten directly. The edible layer that is formed on the surface can actually extend the shelf life by restraining the rate of respiration and microbial growth. Chitosan is a polysaccharide obtained from the deacetylation of chitin [6]. Chitosan has an amine functional group ($–NH_2$) which is positively charged which is very reactive, so that it is able to bind to negative-charged bacterial cell walls [7]. Its strong, elastic, and flexible properties are the advantages of chitosan coatings [8] and its edible nature makes chitosan classified as an environmentally friendly packaging material [9].

The application of increasing the concentration of chitosan in various fishery products has been widely studied before. According to [10], the addition of 2% chitosan gave the best results in red tilapia fillets until the 13^{th} day of storage. According to [11], the addition of 1.5% chitosan and vacuum packaging on presto mackerel is the optimal result until the 14^{th} day of storage. [12], stated that the application of 2% chitosan in rainbow salmon can be stored until the 11^{th} day. According to [13] research, chitosan with a concentration of 2% is the concentration that provides optimal results for storing catfish fillets at low temperatures until the 11th day of storage. Based on the descriptions stated above, the percentage of chitosan that should be added so that it can extend the shelf life of steamed shrimp at low temperature storage is still unknown and needs to be investigated. The purpose of this study was to inhibit

the damage of steamed shrimp which were given chitosan edible coating during the storage period.

2. MATERIALS AND METHODS

2.1 Producing Chitosan

The process of producing chitosan were carried out based on [14] study and was produced through several stages, namely as follows:

a. Deproteination. This process is carried out at a temperature of 60-70 ° C using 1 M NaOH solution with a ratio of shrimp powder to NaOH = 1:10 (g powder/ml NaOH) while stirring for 60 minutes. Then the mixture is separated by filtering and the precipitate is taken.
b. Washing and drying. Washing of sediment was carried out using aquadest until the pH was neutral. Then filtered to take the precipitate and proceed with drying
c. Demineralization. Mineral removal was carried out at a temperature of 25-30 ° C using 1 M HCl solution with a ratio of the sample to HCl = 1:10 (g powder/ml HCl) while stirring for 120 minutes. Then the mixture is separated by filtering and the precipitate is taken.
d. Color removal. The demineralized precipitate was extracted with acetone and blended with 0.315% NaOCl (w/v) for 5 minutes at room temperature. The ratio between solid and solvent used is 1:10 (w/v).
e. Washing and drying. Washing of sediment was carried out using aquadest until the pH was neutral. Then filtering is carried out and the precipitate is dried.
f. Deacetylation of chitin to chitosan. The chitin that has been produced in the above process is put in a 50% NaOH solution at a temperature of 90-100 oC while stirring at a constant speed for 60 minutes. The results obtained are in the form of slurry then filtered and the precipitate is washed with aquadest then aqueous HCl solution is added so that the pH is neutral then dried.

2.2 Edible Coating Solution

This study consist of several treatments based on chitosan coating addition percentage. The coating solution is made by dissolving the ingredients listed in the formulation (Table 1.). The formulation for making the edible coating were selected according to [15] study.

Table 1. Chitosan edible coating formulation based on treatments

Material	Concentration (%)				
	A	B	C	D	E
Chitosan	0	1.5	2	2.5	3
Acetic Acid	0	1	1	1	1
Aquadest	0	2	2	2	2
Glycerol	0	1	1	1	1

2.3 Steamed Shrimp Processing

The raw material used for this research was Vanname shrimp (*Litopenaeus vannamei*) in the form of PUD (peeled undevine). The size of the shrimp used was 60-70/kilogram in the form of PUD. Fresh peeled PUD shrimp initially washed using cold water and then steamed at a temperature of 100 ^{o}C for 5 minutes [16]. Afterwards, the shrimp were drained amd subsequently dipped in an edible coating solution, then finally packed and stored at low temperature.

2.4 Edible Coating application

The chitosan-based edible coating were then be applied to the drained steamed shrimp as a coating by dipping the steamed shrimp for as long as 3 minutes time. The immersion time is determined based on [17].

2.5 Packaging and Storage of Coated Steamed Shrimp

Steamed shrimp that have been coated with edible coating will be packed using styrofoam dish (12 x 12 square centimeters) and plastic wrap is used to cover the top surface, therefore the sorrounding air does not easily in contanct with the packed materials. Packaged steamed shrimp are put in the refrigerator and are not stored in stacked position to avoid physical contact between packaged steamed shrimp and other parts. The storage temperature used was 5-10 ^{o}C and observed for a minimum of 12 days of storage.

2.5 Total Plate Count

Observation of the microbiological parameters of the total plate count was carried out based on [18]. This analysis is done by counting the number of bacterial colonies that grow on the culture media. The calculation is carried out using a commonly used method, namely Total Plate Count. Before carrying out the microbiological test, it is necessary to sterilize the equipment to be used. This observation aims to calculate the total colonies of decaying microbes from solid and liquid samples using serial dilution methods and

pouring plates. To report the results of microbiological analysis by means of plate counts, a standard called Standard Plate Counts (SPC) was used as follows:

a. The dishes selected and counted are those containing the colony count between 30 and 300.
b. Several colonies that are joined together constitute one large colony where the number of colonies is doubtful to be counted as one colony.
c. A row of colony chains which appear as a solid line is counted as one colony.

The microbiological test procedure is carried out as follows:

a. A sample of 3 grams which has been mashed first is then put into a bottle containing 27 ml of physiological NaCl, then after that it is shaken until it is homogeneous so that a 10-1 dilution is obtained.
b. The sample solution is piped 1 ml and put in a test tube containing 9 ml of physiological NaCl to obtain a 10-2 dilution, and so on. When finished, the solution is left for 10 minutes.
c. Each dilution was pipetted as much as 1 ml and then was putted into a sterile petri dish.
d. As much as 10 ml Nutrient Agar media was added to sterile petri dishes in order to calculate the total number of microbes. Immediately close the petri dish and then rotate it in a circle or form a figure of eight slowly until the media is evenly distributed.
e. After the agar media freezes, the petri dishes are wrapped in paper and then stored upside down in an incubator at 37^{o}C. The calculation of the number of bacterial colonies was carried out after the incubation period with a colony counter.
f. Total bacterial counts were carried out on days 1, 2, 3, 4, 5, 7, 8, 9, 10, 11 and 12, the count was stopped until the observation was up to the limit of the maximum bacterial count.

2.5 Data Analysis

The research data were analyzed descriptively. Total bacterial colonies were analyzed using the maximum total bacterial colony standards so that they could be allowed to be consumed in a product or foodstuff.

3. RESULTS AND DISCUSSION

The number of bacteria found in a food ingredient is an indicator to determine the level of spoilage of a food ingredient or the limit of a food ingredient to be consumed. The calculation of the number of bacteria in this study was carried out using the duplicate total plate count method. The results of the observations are presented in Table 2.

Table 2. Steamed shrimp bacterial count average based in treatments

Material	Concentration (%)				
	A	B	C	D	E
	Chitosan (0%)	Chitosan (1.5%)	Chitosan (2%)	Chitosan (2.5%)	Chitosan (3%)
1	$5{,}5x10^{2}$	$6{,}9x10^{2}$	$3{,}7x10^{2}$	$8{,}5x10^{2}$	$6{,}8x10^{2}$
3	$4x10^{3}$	-	-	-	-
4	**$3{,}1x10^{5}$**	$8{,}6x10^{2}$	$4{,}8x10^{2}$	$3{,}7x10^{3}$	$7x10^{3}$
5	$4{,}2x10^{6}$	-	-	-	-
7	-	$4{,}1x10^{3}$	$3{,}2x10^{3}$	$3{,}1x10^{4}$	$6{,}4x10^{4}$
8	-	-	-	-	-
9	-	$4{,}4x10^{4}$	$1{,}4x10^{4}$	$5{,}4x10^{4}$	$5{,}9x10^{4}$
10	-	**$5{,}1x10^{5}$**	$1{,}8x10^{5}$	$2{,}7x10^{5}$	$3{,}2x10^{5}$
11	-	$4{,}4x10^{6}$	$2{,}5x10^{5}$	$3{,}4x10^{5}$	$4{,}1x10^{5}$
12	-		**$3{,}6x10^{5}$**	**$4{,}6x10^{5}$**	**$4{,}8x10^{5}$**
13	-		$3{,}1x10^{6}$	$6{,}3x10^{6}$	$9{,}2x10^{6}$

Observation results during low temperature storage in steamed shrimp samples that were not given chitosan edible coating and chitosan edible coating showed that the number of bacteria tended to increase. In general, the increase in the number of bacterial colonies that occur during storage is caused by the growth of these microorganisms which are influenced by time. In addition, the growth of microorganisms can also be influenced by food (nutrition), humidity, temperature, and oxygen content [19]. Bacteria that can live at low temperatures are cryophyllic bacteria because these bacteria can live optimally at 10^{o}C. According to [20], common spoilage microbes in shrimp commodities include *Pseudomonas* spp., *Aeromonas* spp., *Shewanella putrefaciens*, *Carnobacterium* sp., *Photobacterium phosporeum*, *Enterobacteriaceae* and *Bacillus* sp.

Bacterial growth in steamed shrimp samples with chitosan edible coating was slower than steamed shrimp that did not use it. The number of bacteria during storage on steamed shrimp coated with chitosan edible coating ranged from $6.9x10^{2}$ - $9.2x10^{6}$ colony units /gram, while in samples of steamed shrimp that were not coated with chitosan edible coating the numbers ranged from $5.5x10^{2}$ - $4.2x10^{6}$ colonies / gram. The control TPC value on the 1st day was $5.5x10^{2}$, while the TPC values for all treatments on the 1st day

were 6.9x10^2 colonies / gram, 3.7x10^2 colonies / gram, 8.5x10^2 colonies / gram, and 6.8x10^2 colonies. / gram. The TPC value on day 1 was quite high because microbial activity had occurred at the beginning of storage. Steamed shrimp that did not use chitosan edible coating experienced a very high increase in the number of microbes. The acceptance limit for steamed shrimp coated with chitosan edible coating with a chitosan concentration of 1.5% reaches up to the 10th day with a total number of microbes of 5.1x10^5 colonies / gram. While samples with a concentration of 2%, 2.5%, and 3% chitosan reached the 12th day with the number of bacteria 3.6x10^5 colonies / gram, 4.6x10^5 colonies / gram, and 4.8x10^5 colonies / gram.

Edible coating treatment of samples with added chitosan as much as 2%, 2.5%, and 3% showed that the level of bacterial increase tended to be constant. In this study, the addition of 2%, 2.5%, and 3% of chitosan reached the longest shelf life of 12 days. The 2%, 2.5%, and 3% chitosan treatments had acceptance limits on the same day and reached the optimum at the 2% chitosan concentration treatment so that the 2.5% and 3% treatments did not make the shelf life of steamed shrimp longer than chitosan 2 %. The addition of chitosan concentration did not have a significant effect [21]. This is because a high concentration of chitosan will result in a solution that is too thick or forms a gel so that the solution will be difficult to diffuse compared to a more dilute solution [22].

Based on the results of this study, the optimum effectiveness of chitosan as an antimicrobial was at a concentration of 2% addition. This is supported by [13] research which shows that chitosan with a concentration of 2% is the optimal concentration for storing catfish fillets at low temperatures until the 11th day. [12], stated that the application of 2% chitosan as an edible coating shows an antimicrobial, antioxidant effect, and as a barrier to fat oxidation reactions which can extend the shelf life of rainbow salmon at low temperatures until the 11th day of storage.

Samples of steamed shrimp that were not coated with chitosan edible coating with chitosan edible coating could extend the shelf life from 4 days to 12 days. This is similar to the research of [23] which used a coating process on precooked shrimp using Longevitas (Bio-Envelop Technologies Inc.) edible coating base solution combined with gamma ray irradiation and the results can inhibit the growth of bacteria and pathogens, and can extend the shelf life from 3 days to 3 days. 10 days. The standard TPC value for the maximum limit of bacteria in fresh shrimp is 10^5 colony units / gram [24]

The main mechanism of chitosan in inhibiting microbial growth is that chitosan has a positive amino group capable of binding negative carboxylate groups to the surface of bacterial cells [25]. According to [26], the presence of a positive charge on the amino group causes the ion to interact with the negative charge contained in the microbial cell membrane so that it can cause leakage of proteins and intracellular components in microorganisms (Shahidi et al. 1999). The inhibition of bacterial growth by chitosan begins with the meeting of the bacterial cell wall with the chitosan molecule. Chitosan can bind to cell proteins, including glutamate, which is a component of cell membranes. Chitosan also binds with phospholipids in the membrane, causing increased inner membrane permeability. The increased permeability of the inner membrane can facilitate the release of cell fluids which can be accompanied by other cell components. If this phenomenon occurs, it will not cause cell regeneration and can even cause death [27]. According to [28], the increase in the number of bacteria is influenced by intrinsic and extrinsic factors. The intrinsic factor is microbial growth, while the extrinsic factor includes storage temperature. Microbes may not all die because there are microbes that live in the cavities or cells in steamed shrimp. This is because the chitosan solution cannot absorb into or the all spaces of the steamed shrimp, maybe it can only coat the surface of the steamed shrimp.

Conclusion

Based on the results of the research that has been done, it can be concluded that the addition of 2% chitosan to the edible coating gives the best results in storing steamed shrimp at low temperatures. Steamed shrimp coated with 2% chitosan edible coating reached the acceptance limit until the 12th day of storage with the number of bacteria 3.6x10^5 colonies / gram.

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