Application of water soluble chitosan for vannamei shrimp (*Litopenaeus vannamei*) preservation at chilling temperature

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Abstract. Active biomolecules such as chitosan and its derivatives have a role in food products preservation due to its antimicrobial properties. The aim of this study is to determine the ability of water soluble chitosan to extend shelf life of peeled and deveined shrimp stored at the chilling temperature. This study used completely randomized design using three treatments (control, 2% chitosan solution, and 2% water soluble chitosan solution) with six replications. Samples were tested for aerobic plate count, pH, total volatile base nitrogen, and sensory (appearance, odor, and texture). Experiments on water soluble chitosan indicated number of microbes was still within the safe limit until the 6th day. This could be identified from the number of microbes which still could be accepted by consumers (< 5 x 10^4 colonies/gram) at the 6th day, while in the control treatment the microbial amount within the safe limit until the 3rd day. Total Volatile Base Nitrogen of water soluble chitosan treatment was 3.69 mgN/100 grams. The pH value of shrimp treated with water soluble chitosan was 6.8, while in the organoleptic test, water soluble chitosan treatment had significant difference from control (P <0.05). This research conclude that water soluble chitosan have properties that can preserve food products specially vanamei shrimp.

1 Introduction

Shrimp contains more free amino acids such as glutamic acid, aspartic acid, alanine, glycine, valine, threonine, leucine, lysine, tyrosine, serine, histidine, arginine, proline, phenylalanine, isoleucine, methionine, cystine and nitrogenous bases compared to chicken or beef so it requires better treatment such as the addition of food additives in the storage process. Shrimp may suffer quality deterioration during the storage process, and the occurrence of bacterial and enzyme activity [1]. Active biomolecules such as chitosan and its derivatives have a role in food applications as they have antimicrobial properties [2]. Chitosan has great potential to be utilized in various fields such as food and health, but chitosan is not soluble in water at

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neutral pH, limiting its applications in various fields such as food, health, agriculture, and biomedical applications [3].

Although chitosan itself already has good preservation properties for food products, it can only dissolve at acidic pH. This is a shortcoming that must be improved, because food can change its flavor and aroma when mixed with acidic solutions. So, this study aims to prepare water-soluble chitosan at neutral pH that it can be applied in food products such as shrimp preservatives. The method employed in this study is the chemical method with the addition of filler. The function of adding filler is to accelerate the drying process of chitosan and to provide powder form [4, 5]. It is not known how the modified chitosan will perform as a food product preservative application.

2 Materials and methods

2.1 Tools and materials

The equipment the tools used in this study are autoclave (Yamato SM52 Autoclave, Japan), refrigerator (Toshiba GR-M245H Glacio XD7, Japan), pH meter (Eutech pH 700), incubator (Thermolyne type 42000 Incubator, USA), centrifuge (EBA 20, Germany), water bath thermostat (HH-6), analytical balance (Ohauss Pioneer 0-2100 G, USA), oven.

The materials used in this research are vanamei shrimp, chitosan, acetic acid, peroxide acid (H_2O_2), Ethanol (C_2H_5OH), Plate Count Agar (PCA), distilled water, 70% alcohol, cotton pads, methylated spirits, sodium hydroxide (NaOH), hydrochloric acid (HCl), maltodextrin.

2.2 Methods

This research uses an experimental method with a research design that is a Completely Randomised Design (CRD) with 3 treatments and 6 replicates. Vanamei shrimp samples were obtained directly from ponds in the Keputih area, East Surabaya. Samples of shrimp measuring ± 8 cm per head were put into a cool box with ice cubes added that had a temperature of 5 °C during the transportation process to the laboratory.

Ten grams of chitosan was dissolved in 990 ml 1% acetic acid solution. Hydrogen peroxide (H_2O_2) was added to the homogenised chitosan solution as much as 20 ml or 2% of the volume of the chitosan solution. The chitosan solution was then cooled in the refrigerator. The chitosan solution was neutralised using 1 M NaOH, then precipitated with ethanol for 24 hours. The precipitation process uses ethanol as much as 2 times the volume of chitosan solution.

The precipitated solution was then subjected to a centrifuge process to separate the filtrate which was wet water soluble chitosan and the supernatant which was ethanol. Wet water soluble chitosan was then weighed as much as 5 grams in a petri dish and mixed with filler with a concentration of 30%. The mixture was then ovenised at 50 °C for 24 hours. The water soluble chitosan was then pulverised using a grinder until it became powder.

The prawns were peeled and de-headed using a knife. The peeled and de-headed prawns were immersed in 0.1% chitosan solution for five minutes. The soaked shrimp were placed in a plastic bag. The shrimp were stored in a refrigerator at 5 °C for 14 days and observed every 24 hours.

Aerobic Plate Count is used to determine the number of microbes in the product. Equipment that will be used such as erlenmeyers, microtips, test tubes, and petri dishes are sterilised first. The equipment was sterilised for 15 minutes at 121°C.

Plate Count Agar (PCA) media was prepared by dissolving 8.75 grams of PCA powder with 500 ml of distilled water. The solution was heated and homogenised on a heater stirrer. The PCA solution that was already boiling and homogeneous was put into an autoclave for 15 minutes at 121°C.

One gram of sample was dissolved in 9 millilitres of 0.8% NaCl solution. This solution is a 10^{-1} solution. The sample was dissolved to a dilution of 10^{-4}.

The plating method used was spread-plate. PCA medium was poured into petri dishes. 0.01 millilitres of solution of each dilution was moved to a sterile petri dish using a miropipete with a sterile tip. Plating of each dilution was done 3 times.

The number of aerobic bacteria contained in the sample is calculated by the formula:

$$N = \frac{\sum C}{[(1 \times n_1) + (0{,}1 \times n_2) \times (d)]} \quad (1)$$

Notes :
N = number of colonies per ml or per gram of product
ΣC = number of colonies in each petri dish counted
n_1 = number of petri dish in the first dilution counted
n_2 = number of petri dish in the second dilution counted
d = first dilution counted

The pH test was conducted using a pH meter (Eutech pH 700). Five grams of sample was dissolved in 45 millilitres of distilled water. The sample solution was tested by dipping the pH meter electrode. Measurements were taken three times to obtain accurate pH results.

Shrimp samples weighing 10 grams were homogenised and soaked using 100 ml of distilled water for 30 minutes and then filtered. Boric acid as much as 1ml was mixed with 1 drop of methylred and 1 drop of methylene blue in the middle of the Conway cup, then 1 ml of filtrate and 1 ml of potassium carbonate solution was added to the edge of the Conway cup and mixed. Conway cup was closed and incubated at 37°C for 2 hours. The incubated solution was titrated with 0.01 mol/litre HCl. The formula used to determine TVBN levels is as follows:

$$\text{TVBN} = (T_{\text{treatment}} - T_{\text{blanco}}) \times \text{acid concetration} \times 0{,}2 \times V1/V2 \times 100/W \quad (2)$$

Hedonic testing used a 9-point scale, ranging from one strongly disliked to 9 strongly liked. Panelists were asked to rate the characteristics of the prawns. Characteristics assessed included colour, aroma and texture. Data were analysed using SPSS 16 with a significance value of $p<0.05$.

Statistical calculations were carried out with SPSS 21 software. In the organoleptic test, the kruskal-wallis method was used. In other tests, anova test was used with Duncan's further test. Calculations were made to see whether there was an interaction between the amount of ALT and pH with immersion using a water-soluble chitosan solution containing 30% and 40% maltodextrin filler, acetic acid, and distilled water.

The parameter in this study was the deterioration of shrimp quality stored at chilling temperature. Quality deterioration was measured by TVBN, APC, pH, and sensory tests.

3 Results and discussion

In this organoleptic test, thirty panellists were used. The average value of shrimp organoleptic is listed in Table 1. The Kruskal-Wallis test results showed that the appearance and aroma parameters had values of 0.002, 0.001 and 0.002, respectively. All parameters have a value of ($P<0.05$). The final result of the Kruskall Wallis test is the P value, which if the value is <

the critical limit (0.05) then a statistical conclusion can be drawn that water soluble chitosan, chitosan and control have a significant effect on organoleptic parameters (appearance, aroma and texture).

Table 1. Average organoleptic score.

Treatment	Appearance	Aroma	Texture
Water Soluble Chotosan	6.7611 ± 0.2984	6.4722 ±0.2068	5.9500 ± 0.3007
Chitosan	6.0556 ±0.2543	5.6667 ± 0.1139	6.2000 ± 0.1069
Control	5.8389 ±0.1626	6.0833 ± 0.1134	5.7611 ± 0.1145
Kruskal-wallis	0.002	0.001	0.002

Based on the assessment of panellists, shrimp treated with water-soluble chitosan had the highest organoleptic value on the appearance and aroma parameters, but on the texture parameter chitosan had a higher value compared to water-soluble chitosan and control. This can be seen from the Mean Rank value. In the appearance parameter, the mean rank values for water soluble chitosan, chitosan and control were 15.50, 8.08 and 4.92, respectively. On aroma parameter, the mean rank values for water soluble chitosan, chitosan and control were 15.33, 3.50 and 9.67, respectively. While on texture parameter the mean rank values for water soluble chitosan, chitosan and control were 8.00, 15.50 and 5.00.

On the appearance parameter, the organoleptic test value of water soluble chitosan was higher than the control. This is due to the water-soluble chitosan solution being able to coagulate mucus on the surface of the shrimp. The polycation properties of water-soluble chitosan can bind to bacterial proteins so as to inhibit bacterial growth [6].

During storage, the change of colour in shrimp may occur due to pigment oxidation and lipolysis. These factors allow for the degradation of acanthine in shrimp [7].

Changes in shrimp colour during storage are caused by lipid oxidation [8]. Water soluble chitosan can penetrate into the shrimp. This can help protect saturated fatty acids that are easily oxidised in shrimp [9].

Based on the organoleptic test results, the texture of the chitosan-treated treatment had a better average value than the other treatments. The hardness of shrimp meat increased with the higher molecular weight of chitosan. The higher the molecular weight of chitosan, the viscosity of the solution will also increase [10].

In the aroma parameter, chitosan treatment had the lowest value. This is because the chitosan treatment used acidic solvents, thus affecting the aroma of vaname shrimp. The chitosan treatment was significantly different from the control and water soluble chitosan treatments ($P<0.05$), but the water soluble chitosan treatment was not significantly different from the control treatment ($P>0.05$).

Table 2. Vaname shrimp pH test results.

Day	Water Soluble Chitosan	Chitosan	Control
0	7 ± 0.08	6.5 ± 0.09	7.2 ± 0.11
2	7 ± 0.09	6.5 ± 0.09	7.1 ± 0.13
4	6.9 ± 0.07	6.5 ± 0.09	7 ± 0.12
6	6.9 ± 0.08	6.5 ± 0.08	6.9 ± 0.11
8	6.9 ± 0.08	6.6 ±0.09	6.9 ± 0.11
10	6.8 ± 0.07	6.6 ± 0.1	6.9 ± 0.12
12	6.8 ± 0.07	6.7 ± 0.08	6.9 ± 0.13
14	6.8 ± 0.09	6.7 ± 0.09	6.9 ± 0.12

Based on the anova test results for the treatment of water-soluble chitosan, chitosan, and control on day 6, it can be concluded that there is no significant difference between the three treatments (P>0.05). On day 14, the treatment of water-soluble chitosan, chitosan and control had no significant difference among the three treatments (P>0.05).

Based on Table 2, the pH of each treatment on day 0 was close to neutral, except for the immersion treatment with chitosan which had a pH value of 6.5. This is due to the increase in acidic components that cause a decrease in pH value [5].

In the process of quality deterioration, the decomposition of nitrogenous components increases the pH of shrimp [11]. The pH of the control shrimp on day 0 was 7.2. But chitosan-treated shrimp had a low pH value of 6.5. This is due to the treatment of chitosan using acidic solvents so that the acid component increases.

Fishery products are acceptable to consumers with pH values up to 6.8, but can be considered spoiled if the pH value is above 7. Consumer acceptance limits are generally in the 6.8-7 range [12].

In chitosan-treated vaname shrimp, the pH value consistently increased during the storage process. This can occur due to the active cathepsin enzyme at acidic pH which causes protein degradation resulting in an increase in pH in shrimp [13].

Whereas in control vaname shrimp and in shrimp treated with water soluble chitosan, the pH decreased consistently. This is due to the shrimp experiencing rigor mortis phase so that the pH drops. Anaerobic respiration causes the accumulation of lactic acid so that the pH drops which indicates the rigor mortis phase has occurred. Anaerobic respiration occurs due to the cessation of blood flow that carries oxygen so that the anaerobic reaction of glycogen that produces lactic acid [14].

Total volatile base nitrogen represents the sum of ammonia and other nitrogenous base components. Tvbn is a test to determine the level of spoilage [15].

Table 3. Average value of total volatile base nitrogen.

TVBN	Control± SD	Chitosan ± SD	Water Soluble Chitosan ± SD
Day-0	0.70 ± 0.253^{a}	0.98 ± 0.310^{a}	0.70 ± 0.253^{a}
Day -1	2.10 ± 0.506^{b}	1.40 ± 0.352^{a}	1.40 ± 0.167^{a}
Day -2	2.94 ± 0.219^{a}	2.05 ± 0.408^{a}	2.01 ± 0.207^{a}
Day -3	3.50 ± 0.179^{b}	2.43 ± 0.513^{a}	2.61 ± 0.082^{a}
Day -4	3.92 ± 0.167^{b}	2.75 ± 0.532^{a}	3.13 ± 0.207^{a}
Day -5	4.25 ± 0.163^{b}	3.13 ± 0.532^{a}	3.46 ± 0.242^{a}
Day -6	4.72 ± 0.327^{b}	3.60 ± 0.547^{a}	3.69 ± 0.393^{a}

Different superscript in the same column indicates significant difference (P<0.05)

Based on the results of the ANOVA test, on day 0 there was no difference in nitrogen levels from each treatment. On day 1 to day 6, chitosan and water soluble chitosan treatments had no difference, but in the control treatment there was a difference.

Based on Table 3, the initial average amount of control TVBN was 0.7 mgN/100 grams, on day 6 it increased to 4.72 mgN/100 grams. The initial average amount of TVBN of chitosan was 0.98 mgN/100 grams, on day 6 it increased to 3.6 mgN/100 grams and the initial average amount of TVBN of water soluble chitosan was 0.7 mgN/100 grams, on day 6 it increased to 3.69 mgN/100 grams. All treatments experienced an increase in the amount of TVBN during the six days of storage.

The TVBN value increased with increasing storage time in all treatments. The highest increase in TVBN value occurred in the control, which was 4.72 mgN/100 grams. This indicates that vaname shrimp treated with chitosan and water soluble chitosan were able to reduce the rate of formation of nitrogenous bases.

The increase in the amount of TVBN is directly proportional to the increase in the number of bacteria. This occurs due to bacterial activity that breaks down macromolecular compounds into simpler compounds. TVBN concentration increases due to the process of protein degradation such as ammonia, histamine, and trimethylamine [15].

The presence of water soluble chitosan treatment on vaname shrimp inhibited bacterial growth during the storage process, causing the less amount of TVBN produced. Based on consumer acceptance standards, all treatments are still in the range that can be accepted by consumers. The amount of TVBN that is still accepted by consumers is ≤ 20 mgN/100 mg. The following are the Aerobic Plate Count test results from shrimp treated with water soluble chitosan, chitosan, and control (Fig. 1).

Aerobic Plate Count test results on day 0 showed that water soluble chitosan and chitosan had the same number of bacterial colonies, namely 2.72 x 10^4 CFU/gram (4,435 log CFU/gram). While the control has more colonies, namely 3.03 x 10^4 CFU/gram (4,481 log CFU/gram).

On day 3 water soluble chitosan had a colony count of 1.5 x 10^4 CFU/gram (5,176 log CFU/gram), chitosan 1.4 x 10^5 CFU/gram (5,146 log CFU/gram), and control 3.09 X 10^5 CFU/gram (5,490 log CFU/gram). On day 6, the number of colonies found in water soluble chitosan, chitosan, and control were 2.9 X 10^5 CFU/gram (5,462 log CFU/gram), 2.7 X 10^5 CFU/gram (5,431 log CFU/gram) and 1.6 X 10^6 CFU/gram (6,204 log CFU/gram), respectively.

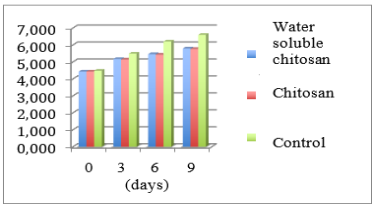

Fig. 1. Aerobic plate count test result (logCFU/gram).

On the 9th day, the number of colonies found in shrimp treated with water soluble chitosan was 6.2 X 10^5 CFU/gram (5,792 log CFU/gram). While the number of colonies found in shrimp treated with chitosan and control were 5.95 X 10^5 CFU/gram (5,771 log CFU/gram) and 3.95 X 10^6 CFU/gram (6,597 log CFU/gram). The number of bacterial colonies that are still acceptable to consumers and still declared safe for consumption is 5.0 X 10^5 CFU/gram [16].

The results of the Aerobic Plate Count (APC) calculation for nine days of storage can be seen in Fig. 1. The initial APC value in each treatment was below 5.0 x 10^4 CFU/gram (4.435 log CFU/gram). This indicates that the quality of the tested vaname shrimp was still in good condition. The APC value in the control treatment exceeded the acceptable limit for consumers on day 6. On day 6, the APC value in the control treatment was 1.6 x 10^5 CFU/gram (6,204 log CFU/gram), far from the requirements that can be accepted by consumers, which is 5.0 x 10^4 CFU/gram [16]. Meanwhile, the APC values of chitosan and water soluble chitosan treatments on day 6 were 2.7 x 10^4 CFU/gram (5,431 log CFU/gram) and 2.9 x 10^4 CFU/gram (5,462 log CFU/gram). On day 9, the APC value in each treatment has exceeded the amount of APC value that is still acceptable to consumers.

Water-soluble chitosan and chitosan have amine groups that can interact with negative groups on the surface of bacterial cells. This causes damage to the cell membrane which results in enlarged pores in the membrane. With the enlargement of the pores in the membrane, the cell membrane cannot regulate the entry and exit of substances from outside and inside the cell. This results in inhibition of metabolic activity in microbial cells. The antibacterial mechanism of chitosan is due to the interaction between protonated amine groups and negative groups on bacterial cell surface components. Chitosan can inhibit

microorganisms by attaching to and disrupting the bacterial cytoplasmic membrane, which causes leakage in the cytoplasm and leads to cell death [2].

In chitosan treatment, acetic acid is used as the solvent. Acid solutions can cause protein denaturation and enzyme inactivation in bacteria, causing disruption of metabolism in bacteria [17].

4 Conclusion

Water soluble chitosan is concluded that it can extend the shelf life of vanamei shrimp in chilling temperature storage. The preservation performance of water soluble chitosan is slightly lower than chitosan but on average of the organoleptic test water soluble chitosan are better.

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