Original Research Article

Effectiveness of Natural Preservative Ingredients Garlic Extract (*Allium sativum*) on The Shelf Life of Fresh Vannamei Shrimp (*Litopenaeus vannamei*)

ABSTRACT

This research aims to determine the best concentration of garlic extract that can be used so that the shelf life of vannamei shrimp can last longer. This research was conducted in May - August 2022 at the Fisheries Product Processing Laboratory, Padjadjaran University. This research used an experimental method. The parameters observed in this research included the phytochemical test of garlic extract, the Total Plate Count (TPC) test, the scoring test and the degree of acidity (pH) test. Statistical of the data obtained from the results of the phytochemical test, Total Plate Count (TPC) and degree of acidity (pH) were analyzed descriptively comparatively while the scoring test was analyzed non-parametrically using Friedman's two-way analysis of variance with the Chi-square test. The results showed that the use of garlic extract with a concentration of 10% on vannamei shrimp during cold storage (0 - 4^{o}C) had the longest shelf life of up to 5 days with a total bacterial count of around 4.9 x 10^{5} cfu / g, a pH value of 7 with the appearance starts to change color, the smell is neutral but starts to smell a little ammonia and the texture is less elastic but still compact and solid.

Keywords: Garlic extract, vannamei shrimp, shelf life, cold temperature.

1. INTRODUCTION

Fresh vannamei shrimp are in great demand in export-import activities to various countries and in the local market [1]. Countries that receive fresh shrimp exports from Indonesia include the United States, the Netherlands, Malaysia, Japan, China, the United Kingdom and Singapore which are even able to absorb as much as 90% of Indonesian shrimp [2]. This can happen because vannamei shrimp have a more savory and sweet taste [3]. The high protein content is 19.38% (Rhodes 2020) compared to tiger prawns which only have 18% protein and giant prawns of 16.5% [4].

Shrimp is included in the perishable food commodity [5]. Decline in the quality of shrimp can be caused by two things, namely a decrease in quality chemically and microbiologically. Chemical degradation can be in the form of enzymatic reactions which can be melanosis or commonly known as blackspots and also oxidation [6]. Melanosis usually occurs on the head and carapace which is due to polyphenoloxidase enzymes and oxidation can occur because the fat in the shrimp reacts with oxygen which causes the shrimp to change color to yellowish

to give off a rotten aroma [7]. Bacterial quality degradation can occur because there are bacteria that attack fresh shrimp such as Vibrio sp., *Clostridium botulinum* type E, *Vibrinus cholera* and *Vibrinus vulvinicus* bacteria [8].

This decline in the quality of the shrimp will have an impact on decreasing sales and creating economic losses because the shrimp look unattractive and not fresh. Several producers use the synthetic chemical preservative sodium metabisulfite to preserve fresh shrimp [9]. According to the Acceptable Daily Intake (ADI) concept introduced by FAO/WHO in 1996, all chemicals used as preservatives are poisons, if the amount used exceeds a predetermined limit and will have an impact on human health [10].

the use of sodium metabisulfite in the preservation of fresh shrimp as much as 2 ppm for 2 minutes and then washing the shrimp with water containing 1-10 ppm of chlorine water. Based on the ADI concept, the consumption dose of sodium metabisulfite permitted by BPOM Regulation in Indonesia No. 11 of 2019 only 0.07 mg/kg human body weight [11]. Health problems caused by the use of sodium metabisulfite is irritation of the mouth tissue [9]. There are other side effects that will arise from the use of sodium metabisulfite, which can trigger allergic reactions, which can include respiratory tract disorders, digestive tract disorders, and skin irritation [12].

Antibacterial compounds are compounds that can inhibit the growth of bacteria. One of the natural ingredients that contain antibacterial compounds is garlic. Garlic contains antibacterial compounds such as essential oils, alkaloids, tannins, saponins and flavonoids and allicin which can slow down the growth and development of bacteria. This is because garlic contains an allicin-like compound of 70-80% [13].

The use of thick garlic extract as a natural preservative has several advantages including from a health perspective, garlic can lower blood cholesterol levels, prevent heart attacks and stabilize a disturbed digestive system and can increase endurance [14]. Another advantage of using thick garlic extract in preservation is that it can reduce expenses because garlic can be cultivated alone and the existence of garlic has been recognized by the wider community as a plant that has various properties.

Garlic extract in its application to food to be preserved, the concentration used must also be considered. Therefore, it is necessary to conduct research to determine the best concentration of condensed garlic extract that can be used to inhibit quality deterioration and extend the shelf life of vannamei shrimp during cold storage (0 - 4^{o}C).

2. MATERIAL AND METHODS

2.1 Time and Place of Research

This research was conducted in June – August 2022. Garlic extract was made at the Central Laboratory of Padjadjaran University and observations and assessments were carried out at the Fishery Products Processing Laboratory, Faculty of Fisheries and Marine Sciences, Padjadjaran University.

2.2 Tools

The research tools used were autoclave, beaker glass, blender, Bunsen, porcelain cup, centrifuge, distillation apparatus, measuring cup, chiffon cloth, cotton and tissue, Erlenmeyer, magnetic stirrer, mortar, oven, petridish, dropper pipette, volume pipette, knife , analytical balance, petri dish, tweezers, pH meter, test tube, spatula, drop plate, measuring cup,

refrigerator, pestle and mortar, schott bottle, laminar, incubator, microtip, micro pipette, pipette, glass rod, hotplate, magnetic stirrer , vortex**.**

2.3 Materials

The research materials used were vanamei shrimp, garlic, 96% ethanol solution, small Styrofoam, plate count agar (PCA), distilled water, masks, gloves, tissues, filter paper, white paper, sterile NaCl, cling wrap, gauze. , buffer solution pH 4 and 7, plastic.

2.4 Research Methods

The method used in this study was an experimental method with 4 treatments of garlic extract and 3 repetitions with concentrations of 0%, 5%, 10% and 15%. Assessment using 20 semi-trained panelists. Panelists used by students of the Faculty of Fisheries and Marine Sciences, Padjadjaran University.

2.4.1 Sterilization of Tools and Materials

Sterilization is the process of removing or killing microorganisms (protozoa, fungi, bacteria, mycoplasma, viruses) in objects or equipment to keep laboratory equipment clean/sterile, and prevent contamination. Objects to be sterilized will be wrapped in brown paper and then put into the autoclave with a temperature of 121 ^{o}C and arranged until neat.

2.4.2 Distillation of Extractive Solvents

Distillation is a method of purifying a mixture and is usually a liquid based on its boiling point and uses a tool called a distillation apparatus [15]. The water pump and heater cable is connected to the electric switch until the power light indicator turns on (1), the solvent is put into the distillation flask (2), the heating button is turned on, the temperature starts to rise (3), the temperature is kept stable according to the boiling point of the solvent (4), the distillate results into the container by rotating the keep (5).

2.4.3 Garlic Extract Preparation

The process of making garlic extract refers to the method of Salim [16] by maceration with a rotary evaporator and using 96% ethanol solvent. The garlic was peeled and then weighed (1), the garlic was sliced (2), the onion was put in an oven that had a blower at 40 ^{o}C (3), the dried garlic was then blended (4), the dried garlic was macerated for 3x24 hours using 96% ethanol solvent, every 24 hours the filtrate is filtered and replaced with a new solvent (5), the garlic solution is filtered using filter paper (6), the filtrate is evaporated using a vacuum evaporator with a speed of 5000 rpm and a temperature of 40 ^{o}C.

2.4.4 Application of Garlic Extract on Shrimp

Extract dilution was carried out according to Nurfitri's research formula [17].

$$M1.V1 = M2.V2$$

Description:

M1 : molarity before dilution
M2 : molarity after dilution
V1 : volume before dilution

V2 : volume after dilution

Shrimps from the coolbox are taken out (1), Prawns that have been treated are stored in styrofoam dishes (2), packed using clingwrap (3), Shrimp are stored in the refrigerator with a temperature of 0 - 4^{o}C and observed (4).

2.5 Parameters Observation

Parameters observed in the study regarding the storability of vannamei shrimp included the garlic extract phytochemical screening test, Microbial count use the Total Plate Count (TPC) test, the scoring test and the pH level test.

2.5.1 Garlic Extract Phytochemical Screening Test

Phytochemical test is a test method used to determine the active compounds contained in plants so that they can be used as treatment [19]. Phytochemical test using Harborne method [20] which includes alkaloids, flavonoids, tannins, saponins, steroids

2.5.2 Microbial Count

The calculation of the number of bacterial colonies was carried out using the Total Plate Count (TPC) method. Fresh shrimp is said to be unacceptable if it exceeds 5x10^{5} cfu / g. Calculation of Total Plate Count (TPC) can be calculated by the following equation (BSN 2015):

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

Description:

N : Number of colonies of the product (colonies/g)
∑C : The number of colonies in all counted plates
n_1 : Number of cups in the first calculated dilution
n_2 : The number of cups in the calculated second dilution
d : First calculated dilution

2.5.3 Skoring Test

The organoleptic test used by the authors in this study was a scoring test using 20 semi-trained panelists. Panelists will be given 4 samples of shrimp that have been given each treatment with the criteria tested including appearance, smell and mucus on the entire surface of the shrimp. The assessment sheet used refers to SNI No. 01-2346-2006 [11].

2.5.4 pH Level Test

The procedure for testing the degree of acidity (pH) is carried out using a pH meter and refers to the AOAC method [22].

2.6 Data Analysis

Data analysis for the results of the garlic extract phytochemical screening test Total Plate Count (TPC), degree of acidity (pH) was carried out in a comparative descriptive manner supported by tables. Non-parametric analysis was performed for scoring testing using

Friedman's two-way analysis of variance with the Chi-square test [18]. Friedman test with the following formula:

$$Xr^2 = \frac{12}{bk(k+1)} \sum_{t-1}^{x} (Rj)^2 - 3b(k+1)$$

Description:

X^2 : Statistic Friedman Test
b : Repetition
k : Treatment
Rj : Total ranking of each treatment

If there are the same numbers, the correction factor (FK) is calculated using the following formula:

$$FK = 1 - \frac{\sum T}{bk\,(k^2 - 1)}$$

Description:

T : N (t^3- t)
t : The number of observations that are the same for one rank
N : The number of the same observation values for one rank with the same t value

Multiple comparison test is defined by the following formula::

$$|Ri - Rj| \leq Z\{\propto / k(K-1)\}\sqrt{bk(k+1)/6}$$

Description:

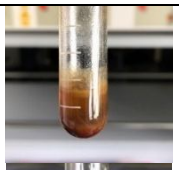
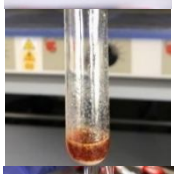
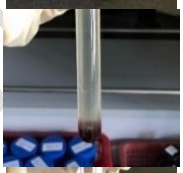
Ri – Rj = Difference in average ranking
Ri = Average rating from sample to i
Rj = Average rating from sample to j
α = Experiment wise error rate at 0,05
b = Amount of combined observation data
k = Number of treatments
Z = Values in Table Z for Multiple Comparison

3. RESULT AND DISCUSSION

3.1 Garlic Extract Phytochemical Screening

Phytochemical screening test was carried out to determine the content of active compounds contained in garlic extract (*Allium sativum*). Based on the results of the phytochemical screening test, garlic extract showed positive for containing several active compounds such as alkaloids, flavonoids, saponins and tannins. Garlic extract contains various secondary metabolites such as alkaloids, flavonoids, glycosides, saponins, steroids, phenols, interquinnon and tannins [23].

Table 1. Garlic Extract Phytochemical Screening Results

Compound	Phytochemical test results	Result	Information
Alkaloid	Brownish yellow and there is a precipitate	+	
Flavonoid	Yellowish red color	+	
Saponin	There is foam as high as 5 cm	+	
Steroid	Not dark purple, the color obtained is reddish	-	
Tanin	Blackish blue in color	+	

The test results stated that garlic extract did not contain steroids. Steroids will be positive if the extract uses a non-polar solvent [11]. Differences in yield can also occur due to the garlic varieties used, temperature, humidity, mineral content and garlic cultivation [25].

3.2 Microbial Count

SNI No. 01-2729.1-2006 regarding the maximum limit of microbial contamination in food, the number of bacterial colonies in food that can be consumed is 5 x 10^5 cfu / g, so if the colonies reach 10^6 cfu / g, shrimp are unfit for human consumption [25].

Table 2. Average Total Number of Bacterial Colonies (cfu / g) of Fresh Vaname Shrimp Based on Garlic Extract Concentration During Cold Storage (0 – 4 ^oC)

Day of Storage	Garlic Extract Concentration (%)			
	0%	5%	10%	15%
0	2,6 x 10^3	2,7 x 10^3	2,8 x 10^3	2,7 x 10^3
2	1,6 x 10^5	2,6 x 10^4	1,9 x 10^4	2,4 x 10^4
4	**4,1 x 10^7**	2,5 x 10^5	2,1 x 10^5	2,5 x 10^5
5	3,0 x 10^8	**3,7 x 10^6**	3,0 x 10^5	5,0 x 10^5
6	9,0 x 10^8	2,3 x 10^7	4,9 x 10^5	**5,3 x 10^6**
7	-	4,0 x 10^7	**9,2 x 10^6**	8,2 x 10^6

8	-	$8,8 \times 10^7$	$1,8 \times 10^7$	$4,7 \times 10^7$
9	-	$5,9 \times 10^8$	$4,8 \times 10^7$	$2,3 \times 10^8$

Based on the observations on day 0, it can be seen that in all treatments the number of bacteria was still below the acceptance limit, which ranged from 2.6 to 2.8 10^3. This was because the bacteria were still in the lag or adaptation phase where the bacteria were still adapting to the new environment so that the have not split [26]. Bacteria are also still adapting to temperature differences and the presence of antimicrobial compounds in vannamei shrimp derived from garlic extract.

The results of the observation on day 2 of storage, the number of vannamei shrimp bacteria treated with garlic extract 5%, 10% and 15% had a number of bacteria respectively 2.6×10^4 cfu / g, 1.9×10^4 cfu / g and 2 .4 cfu / g, the number of bacteria was less than that of the control vannamei shrimp, which was 1.6×10^5 cfu / g.

3.3 Skoring Test

Indicators of organoleptic changes in fresh fishery products including fresh shrimp can generally be tested by scoring tests using a scoresheet based on SNI 2006.

3.3.1 Appearance

Appearance is one of the important things related to the quality of a product. The appearance of fresh shrimp has the characteristics of being whole, bright in color and has a specific aroma (BSN 2006).

Table 3. Median Score Observation Results of Fresh Vaname Shrimp Appearance in Cold Storage (0 – 4 ^oC)

Garlic Extract Concentration (%)	Median Value of Fresh Vannamei Shrimp Appearance in Cold Storage (Day-to)							
	0	2	4	5	6	7	8	9
0%	9	7,5	5	3	1	-	-	-
5%	9	8	7	6	5	3	3	1
10%	9	8	8	7	5	3	3	1
15%	9	8	8	7	5	4	3	1

Note: (-) = no observations were made

The results of observations on the appearance of fresh vannamei shrimp on day 0, namely intact translucent and between the segments are sturdy, while at the time of rejection below the value 7 the appearance of the vannamei shrimp appears to be dull and slightly pink in color, there are black stains and the joints are less sturdy. The appearance value of vannamei shrimp treated with garlic extract with different concentrations was still higher compared to vannamei shrimp without garlic extract treatment (control). This can happen because of the content of phenolic compounds, antibacterial compounds and other antioxidants contained in garlic extract.

The acceptance limit of the panelist results of the appearance scoring test on vaname shrimp was treated with a concentration of 0% (control), namely on day 2. Vannamei shrimp with a concentration of 5% garlic extract reached the acceptance limit on day 4, vannamei shrimp with a concentration of 10% garlic extract reached the acceptance limit on 6 day and

vannamei shrimp with a concentration of 15% garlic extract reached the acceptance limit on 5 day.

Table 4. Average Ranking Value on Vaname Shrimp Appearance

Concentration (%)	Average
0	33a
5	37b
10	53b
15	77b

Note: The average value followed by the same letter indicates that the treatment is not significantly different with a test level of 5%.

Based on the statistical test results, the appearance of vannamei shrimp at 0% concentration treatment (without garlic extract treatment) was significantly different from the appearance of vannamei shrimp with extract treatment at concentrations of 5%, 10% and 15%, which means that the appearance of shrimp at 0% concentration treatment gave results not good compared to the appearance of vannamei shrimp with extract treatment with concentrations of 5%, 10% and 15%. Based on the results of the organoleptic scoring assessment, it can be seen that the treatment with extracts at concentrations of 5%, 10% and 15% had a significant effect on appearance compared to the control treatment.

Table 5. Median Score Observation Results of Fresh Vaname Shrimp Appearance in Cold Storage (0 – 4 ^{o}C)

Garlic Extract Concentration (%)	Median Value of Fresh Vannamei Shrimp Appearance in Cold Storage (Day-to)							
	0	2	4	5	6	7	8	9
0%	8,5	7	5	3	1	-	-	-
5%	9	8	7	5	5	3	1	1
10%	9	8	7	7	6	5	3	1
15%	8,5	8	7	7	4	4	3	1

Note: (-) = no observations were made

the organoleptic value of vaname shrimp aroma with a concentration of 10% and 15% was still acceptable until the 5th day, while the shrimp treated with 5% extract was acceptable until the 4 day. Vaname shrimp that were not given garlic extract treatment had a limit of aroma acceptance until day 2, with a median value of 7, which started to smell a different aroma, such as the smell of ammonia. The results of preserving vannamei shrimp with 5% and 10% garlic extract treatment did not change the resulting aroma to garlic aroma during observation.

3.3.2 Aroma

Bacterial activity in fresh shrimp can reduce the quality of the shrimp as indicated by changes in aroma and even the decomposition process occurs [27].

Table 6. Median Score Observation Results of Fresh Vaname Shrimp Aroma in Cold Storage (0 – 4 ^{o}C)

Garlic Extract Concentration	Median Value of Fresh Vannamei Shrimp Aroma in Cold Storage (Day-to)

(%)	0	2	4	5	6	7	8	9
0%	8,5	7	5	3	1	-	-	-
5%	9	8	7	5	5	3	1	1
10%	9	8	7	7	6	5	3	1
15%	8,5	8	7	7	4	4	3	1

Note: (-) = no observations were made

The median average value of aroma in fresh shrimp during observation ranged from 8.5 to 7 as the panelist's acceptance limit. showed that the organoleptic value of vaname shrimp aroma with a concentration of 10% and 15% was still acceptable until 5 day, while the shrimp treated with 5% extract was acceptable until 4 day. Vaname shrimp that were not given garlic extract treatment had a limit of aroma acceptance until day 2, with a median value of 7, which started to smell a different aroma, such as the smell of ammonia. The results of preserving vannamei shrimp with 5% and 10% garlic extract treatment did not change the resulting aroma to garlic aroma during observation.

Table 7. Average Ranking Value on Aroma of Vaname Shrimp

Concentration (%)	Average
0	31a
5	32b
10	56,5ab
15	76,5b

Note: The average value followed by the same letter indicates that the treatment is not significantly different from a test level of 5%.

Based on the statistical test results, the aroma of vannamei shrimp with 0% concentration treatment (without garlic extract treatment) was significantly different from the aroma of vannamei shrimp with extract treatment with concentrations of 5%, 10% and 15%, which means the aroma of shrimp in the 0% concentration treatment gave the result is a more pungent ammonia aroma compared to the aroma of vannamei shrimp with extract treatment with concentrations of 5%, 10% and 15%. Based on the results of the organoleptic scoring assessment, it can be seen that the treatment with extracts with concentrations of 5%, 10% and 15% had a significant effect on aroma compared to the control treatment.

3.3.3 Texture

The texture parameter is closely related to the observed elasticity of fresh shrimp. In fresh conditions, the texture of the shrimp will be very elastic, compact and dense. The texture of the shrimp will continue to decrease until it is rotten which is marked by the softness of the meat.

Table 8. Median Observation Results of Fresh Vannamei Shrimp Texture in Cold Storage (0-4^{o}C)

Garlic Extract Concentration	Median Value of Fresh Vannamei Shrimp Texture in Cold Storage (Day-to)							
(%)	0	2	4	5	6	7	8	9
0%	9	8	6	3	1	-	-	-
5%	9	8	7	5	5	3	1	1
10%	9	8	8	7	6	5	3	3
15%	9	8	8	7	5	4	3	1

Note: (-) = no observations were made

Based on the results of observations, shrimp treated with 5% garlic extract concentration had a shelf life of up to 4th day with the meat texture starting to become inelastic, shrimp treated with 10% and 15% garlic extract concentrations had an acceptance limit of up to 5 day, while shrimp without garlic extract treatment (control) only until 2 day. There is a decline in quality in shrimp, one of which is marked by the appearance of changes in texture. Contraction of muscle fibers occurs in the rigor mortis phase [25].

Table 9. Average Ranking Value on Vaname Shrimp Texture

Concentration (%)	Average
0	31a
5	36a
10	55,5ab
15	77,5b

Note: The average value followed by the same letter indicates that the treatment is not significantly different from a test level of 5%.

Based on the statistical test results, the texture of the vannamei shrimp with 0% concentration treatment (without garlic extract treatment) was significantly different from the texture of the vannamei shrimp with extract treatment with concentrations of 5%, 10% and 15%. the texture of the shrimp in the 0% concentration treatment gave a softer texture compared to the texture of the vannamei shrimp with the extract treatment with concentrations of 5%, 10% and 15%. Based on the results of the organoleptic scoring assessment, it can be seen that the treatment with extracts at concentrations of 5%, 10% and 15% had a significant effect on texture compared to the control treatment.

3.4 pH Level Test

Testing the degree of acidity (pH) is carried out to determine the level of acidity and alkalinity in a product. The degree of acidity (pH) value can be used as an indicator to determine the level of freshness of shrimp.

Table 10. Average pH Value of Fresh Vaname Shrimp During Cold Storage (0 – 4 ^{o}C)

Day of Storage	pH Score			
	0% (Control)	5%	10%	15%
0	6,7	6,65	6,5	6,6
2	6,3	6,6	6,3	6,4
4	7	6,8	6,5	6,7
5	7,3	6,9	6,9	7,2
6	7,8	7,1	7	7,5
7	-	7,1	7,5	7,7
8	-	7,4	7,6	7,8
9	-	8,1	7,8	8,1

The pH value of shrimp during the cold storage period presented in Table 10 ranges from 6.3 to 8.1. All treatments experienced a decrease in pH value first and then it would increase again. a decrease in the pH value occurs due to the process of autolysis and breakdown of glycogen which produces lactic acid which affects the acidity of the shrimp meat so that the pH value of the shrimp meat will decrease [27]. The longer storage time makes the pH value of the shrimp meat increase again due to the fast metabolic enzymes working in the shrimp meat and also the activity of bacterial growth [24].

On the second day of storage, the pH value of the shrimp without extract treatment (0% or control) and the shrimp with the extract treatment (5%, 10%, 15%) decreased and the pH value of the shrimp meat increased again on the fourth day whether given garlic extract dyeing treatment (5%, 10%, 15%) or without garlic extract dyeing treatment. The increase in pH continued until day 9 which was the last day of observation.

The increase in pH value of shrimp without garlic extract dipping treatment (0% or control) with the addition of garlic extract (5%, 10%, 15%) was faster because garlic extract has several antimicrobial compounds. Tannins are included as antibacterial compounds which when reacted with proteins will form insoluble compounds and cause the proteins in shrimp meat that contain tannins to be difficult to break down so that the results of the reformation which are usually alkaline in nature will take longer to produce [31].

Based on the observations it can be concluded that the pH value of the shrimp without treatment (0% or control) and with the treatment the pH value that has the best characteristics is the garlic extract dipping treatment with a concentration of 10% because it has the lowest decreasing value and the longest time to rise again.

4. CONCLUSION

Based on the research results, it can be concluded that garlic extract with a concentration of 10% is the best concentration for preserving vannamei shrimp based on the longest shelf life, namely until the 6 days with a total bacterial count of 4.9×10^5 cfu / g and a pH value of 7.

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