The Effect of Java Plum Leaf Extract (Syzygium cumini) on Vanname Shrimp Quality (Litopenaeus vannamei) During Cold Storage

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Abstract. L. vannamei is an important commodity in Indonesia. L. vannamei has high nutrition, value especially protein for 35.69% which. Due to high nutritional value the L. vannamei very susceptible for rapid deterioration and need proper handling. S. cumini leaf contain flavonoid compounds, alkaloids, phenols and tannins that can be used as antibacterials. The purpose of this study was to determine the concentration of S. cumini leaf extract and the effect of addition best concentration from S. cumini leaf extract on the quality of L. vannamei during cold storage. The study was experimental laboratory with Completely Randomized Design used factorial pattern with 3 replications. Two factors include storage time (0, 4, 8 and 12) days and second factor was java plum leaf extract concentration (0%, 15%). L. vannamei soaked in 15% java plum leaves extract for 2 hours, then stored in cold temperature ± 4 °C, observed every 4 days. Parameters evaluated include Total Plate Count, Total Volatile Base Nitrogen, pH, Blackspot and Organoleptic. The results showed that L. vannamei with 15% java plum leaves extract during cold storage has significant effect on Total Plate Count, TVBN, pH, Blackspot, and Organoleptic. Value from Total Plate Count test was (5.5 x 10^5 Cfu/g), TVBN value was (35.80 ± 0.21 mgN%), pH value was (8.00 ± 0.24), Blackspot value was (8.60 ± 0.22), and the best overall acceptan value of Organoleptic was (6.21 ± 0.39). The addition of 15% java plum leaf extract can prolong shelf life L. vannamei up to days storage, while the control L. vannamei have been rejected after 4 day of storage.

1. Introduction
The alternative to extended the shelf life of L. vannamei can be done by utilizing natural ingredients namely S. cumini leaves. There have been several studies of S. cumini leaves that can be used to inhibit pathogenic bacteria such as Escherichia coli and Staphylococcus aureus Sudarmi et. al [1]. S. cumini leaves natural preservatif for fisheries product is not yet popular in the community, The S. cumini leaves contain flavonoid compounds, alkaloids, and phenols Gafur et. al [2]. S. cumini leaves contain compounds that can function as antibacterial and antioxidant. These compounds are expected to inhibit the growth of bacteria and prevent the emergence of blackspots quickly, Gowri and Vasantha [3] stated that the results of phytochemical test of S. cumini leaves prove that the leaf contains flavonoid, alkaloid, glycoside, phenol, saponin, and tannin. Flavonoids are useful as antibacterials while phenols can be useful as antioxidants. According to Ramadhani et. al [4], all parts of S. cumini leaves can be used as traditional medicine, but the most bioactive parts are leaves and stems. S. cumini leaves have been studied in vitro to produce some secondary metabolites that can act as antibacterial, allergy, and antioxidants.

Indonesian National Standardization Agency [5], oneway to prevent the emergence of blackspots on shrimp is to use sulfite as an antioxidant. However, sulfites cause allergic reactions in people who are sensitive to these compounds. With the addition of S. cumini leaves is expected to prevent the growth of bacteria and inhibit the work of enzymes that can cause blackspot.

2. Research Methods
2.1. Sample extraction (fitrial and khotimah, 2017)
The research stages were started by cleaning S. cumini leaves with water content. After dry the leaves
are blended and filtered. S. cumini extract was dissolved in the aquaest with the ratio of extract; the
aquaest was 1: 3. Fresh shrimp was soaked in aquaest (control) and 15% java plum leaf extract
solution for 2 hours. Then drained and stored for 12 days at 4 °C cold. Tested TPC (Total Plate
Count), TVBN (Total Volatile Base Nitrogen), pH, Blackspot, and Organoleptic were performed with
observation intervals (0, 4, 8, and 12 days).

2.2. Test procedure
Analysis of Total Plate Count refersh to National Standardization Body Awith procedure, total of 10 g
of sample added 90 ml of BFP solution (Butterfield's Buffered Phospate). The solution obtained is 10-
1 dilution. Dilution 10-2 was obtained by taking 1 ml of solution 10-1. The diluted solution is taken as
1 ml and is inserted in a petri dish containing sterilized cold liquid. Then form the number 8 to flatten
and incubate at 37 ° C for 48 hours [6]; Total Volatile Base Nitrogen, sample of 10 g was blended and
then added with 90 ml of perchloric acid 6%. The sample homogenized and then filtered. The obtained
filtrate is fed into a distillation tube and coupled with a phenolphthalein and silicon anti-foaming
indicator of 2-3 drops each. The sample is added with 10 ml of 20% NaOH and the color turns pink. A
total of 100 ml of 3% H3BO3 were included in Erlenmeyer and Tashiro added 3 drops and the color
changed purple. The sample is distilled until the volume reaches 200 ml and the solution is green.
The sample was titrated with 0.02 N HCl to a purple red. [7]: Level of pH, A sample of 10 grams was
dissolved in 18 ml of aquaest. The electrodes are inserted on the surface of the solution until they are
half liquid. The test is done 2 times each sample.

[8] ; Blackspot refersh to Manheem et. al , The shrimp samples taken are placed in a bright space
then compared with the color measurement scroesheet to describe the development of melanosis.
Tested after shrimp is stored for 1-2 days at cold temperature or room temperature. [9]; and
Organoleptic, procedure is Organoleptic test was performed with 30 samples, 1 sample for 1 panel.
Organoleptic test performed by panelists by groping texture, smell the smell and see the color of
shrimp provided. [10].

3. Results and Discussion
3.1. Total plate count test of L. vannamei
The bacterial count of L. vannamei added with java plum extract are analyzed with total plate count test. The
results of TPC (Total Plate Count) Test of L. vannamei is presented in Table 1.

| Storage at Cold Temperature 4 °C | Treatment | 0% (Control) | 15% (Treatment) |
|---------------------------------|-----------|--------------|-----------------|
| Days 0                          | 0.98 x 10^4 ± 0.11^b | 0.69 x 10^3 ± 0.23^a |
| Days-4                          | 2.10 x 10^5 ± 0.78^d | 1.50 x 10^3 ± 0.53^c |
| Days-8                          | 5.10 x 10^3 ± 0.56^f | 3.10 x 10^2 ± 0.89^g |
| Days-12                         | 7.90 x 10^2 ± 0.98^e | 5.20 x 10^2 ± 0.20^f |

Note: The data is the average result of three times ± standard deviation. Different superscript showed significantly different (p <0.05)

The duration of storage had a significant effect on the growth of bacterial counts contained in L.
vannaei from both treatments. Both treatments at the beginning of storage continue to increase until
day 12. At the beginning of storage, the bacterial count in the shrimp is different, the control treatment
was 0.98 x 10^3 CFU / g and treatment with S. cumini leaf extract was 0.69 x 10^2 CFU / g [22]. L.
vannaei without preservative S. cumini on day 8 storage with TPC value of 5.10 x 10^2 CFU / g, was
not feasible for consumption. The standard allowed on fresh shrimp by SNI (Indonesian National Standard) TPC values of $5.00 \times 10^5$ CFU / g [6].

Flavonoids have several benefits such as anti-cancer, anti-bacterial, anti-inflammatory and anti-allergic. Flavonoids often act as reducing compounds that inhibit many oxidation reactions, both enzymes and non-enzymes Gunalan et.al [11]. Flavonoids are the most numerous compounds in S. cumini leaf. This is strengthened by Utami and Desti [12], which states that flavonoids act as antibacterial by forming complex compounds against proteins from outside the cells that interfere with the strength of bacterial cell membranes.

According to Maharani et. Al [13], alkaloids are compounds possessing the ability as antibacterials. The mechanism of alkaloids as an antibacterial is by interfering with the peptidoglycan component of the bacterial cell, so that the cell wall layer is not completely formed and causes the cell's death.

3.2 Total volatile base nitrogen of L. vannamei

The TVBN (total volatile base nitrogen) value of L. Vannamei increased with the storage time. The TVBN control was higher than sample added with S. cumini (p <0.05). The results of the Total Volatile Base Nitrogen Test of Vaname Shrimp (L. vannamei) is presented in Table 2.

| Old Storage at Cold Temperature | 0% (Control) | 0% (Control) |
|--------------------------------|--------------|--------------|
| Days 0                         | 15.96 ± 0.22b| 14.26 ± 0.80a|
| Days 4                         | 25.82 ± 0.91d| 18.73 ± 0.21c|
| Days 8                         | 36.08 ± 0.11f| 28.01 ± 0.27e|
| Days 12                        | 43.35 ± 0.28g| 35.80 ± 0.23f|

Note:
- The data is the average result of three times ± standard deviation.
- Different superscript showed significantly different (p <0.05)

The length of storage has a significant effect on TVBN values increment on L. vannamei from both treatments. Both treatment at the beginning of storage continued to increase until day 12. In the beginning of storage value of TVBN on L. vannamei is different in the control of TVBN value of 15.96 mgN% and treatment with S. cumini leaf extract value of TVBN of 14.26 mgN%. L. vannamei without preservation (control) was not feasible for consumption at day 8 storage, whereas in addition treatment S. cumini leaf of L. vannamei was not feasible for consumption at day 12 storage. On that day, the control treatment has TVBN value above the standard allowed by SNI (Indonesian National Standard) with an average TVBN value of 30 mgN% [7]. L. vannamei with the addition of java plum leaves extract as much as 15% able to maintain the quality until day 8 with an average TVBN value of 28.01 mgN%. According to Goncalves and Junior [14], which states that shrimp can be said to be fresh if the value of TVB <20 mgN%. Based on the limits of TVB value of shrimp that is still feasible and can be consumed in the phase of pre rigor and rigor mortis. While the post-shrimp rigor phase is not feasible to be consumed due to TVB value> 20 mgN%.

Tannins can serve to prevent protein damage. This is strengthened by Cahyani [15], a compound capable of protecting proteins from bacterial degradation is tannin. This is because tannins are able to bind proteins by forming complex compounds that are resistant to proteases.

3.3 pH test

The pH gradually increased in both sample during the storage. The results pH test of Vaname Shrimp (L. vannamei) is presented in Figure 1.
Figure 1. The pH value of *L. vannamei* from day 0 to 12 in the storage

The longer the storage had a real effect on the increase of pH value found in *L. vannamei* from both treatments. Both treatments at the beginning of storage continued to increase until day 12. At the beginning of storage pH value in different *L. vannamei* that is on the control of pH value of 6.30 and treatment with *S. cumini* leaf extract pH value of 6.00. This is in line with the increasing value of TPC and TVBN. On that day the control treatment had a pH value of 7.80, the figure indicating that the *L. vannamei* on the 8th day without treatment was alkaline. *L. vannamei* with the addition of *S. cumini* leaves extract as much as 15% able to maintain pH until day 8 with average pH value of 7.30. The longer the storage the higher the pH value of a product. This is due to the process of autolysis and bacterial activity. Changes in pH value in the quality deterioration phase can also be due to the production of lactic acid from the decomposition of glycogen in shrimp meat.

Increased pH value along with increasing TVBN value and TPC value on *L. vannamei*. The increase in pH value during storage is due to the degradation of proteins that produce simpler nitrogen compounds. Such compounds include free amino acids and nitrogenous bases. Increased nitrogen bases also cause a rise in pH, due to the formation of amines by the amino acid decarboxylase. ATP degradation is an enzymatic process. While enzyme activity is the cause of pH change. Changes in pH on shrimp are different from fish in general. According to Agustini [16], ATP for invertebrates has a slightly different pattern with fish. The quality of freshness of the fish is affected by the degradation of ATP. Shrimp and shellfish are more dominant to produce AdR than IMP. This difference causes the freshness of shrimp and shellfish not to increase significantly like the fish in general.

3.4. Blackspot Test
The results Blackspot Test of (*L. vannamei*) is presented in Figure 2.

Figure 2. The blackspot of *L. vannamei* from day 0 to 12 in the storage
Key: 0=absent; 2=slight (up to 20%) of shrimps surface affected); 4=moderate (20 to 40% of shrimps surface affected); 6=notable (40 to 60% of shrimps surface affected); 8=severe (60 to 80% of shrimps surface affected); 10=extremely heavy (80 to 100% of shrimps surface affected)
The length of storage gives a real effect on the emergence of blackspot presence in *L. vannamei* from both treatments. Both treatments at the beginning of storage continue to increase until day 12. At the beginning of storage value of blackspot on *L. vannamei* is not significantly different where the shrimp body has not found black spots or blackspot. Blackspots start appearing on shrimp samples on the 4th day. *L. vannamei* with the addition of *S. cumini* leaves extract as much as 15% able to slow the emergence of blackspot compared with *L. vannamei* without treatment (control). According to Manheem *et. al* [9], the limit of consumer acceptance value for blackspots is 4.00. Therefore, the *L. vannamei* soaked in *S. cumini* leaf extract is still acceptable by consumers on the 8th day while the control *L. vannamei* is only accepted until the 4th day. This is because the damage that first occurs after the dead shrimp is a change in the work of the enzyme. According to Perceka *et. al* [17], quality damage to other enzymatic *L. vannamei* is the occurrence of discoloration process or commonly called blackspot or melanosis. The process of melanosis occurs naturally when the shrimp undergo post mortem stages.

According to Benjakul *et al* [18], a constraint that is often found in post-harvest shrimp process is the emergence of black spots or often called a blackspot. Blackspot appears on the segments of the shrimp abdomen. Blackspot indicates that the process of degradation caused by the enzymatic oxidation process.

3.5. Test organoleptic of *L. vannamei*

The organoleptic value decreased over the long storage period. The results Organoleptic Test of (*L. vannamei*) is presented in Figure 3.

![Figure 3. The organoleptic value of *L. vannamei* from day 0 to 12 in the storage](image)

The longer storage time can decreased the organoleptic value of the shrimp. In the shrimp samples given the addition of *S. cumini* leaf extract at 15%, the shrimp are still received by panelists until day 8. While on samples without treatment of organoleptic value of shrimp on the 8th day has been rejected by panelists. This is due to changes in enzymes and the growth of bacteria that cause shrimp decay.

According to the National Standardization Agency [10], the shrimp that can be consumed for sensory parameters is at least 7. While the shrimp vans with 8 days storage (control treatment) and 12 days (15% concentration treatment) are unacceptable by panelists and incompatible with standard fresh shrimp because it has a value less than 7.

4. Conclusion

The conclusion from this research is the concentration of 15% *S. cumini* leaf extract can be used to maintain the quality of shrimp vaname (*L. vannamei*) and *S. cumini* leaf extract able to maintain the quality of vaname shrimp stored at low temperature from TPC, TVBN, pH, Blackspot and organoleptic tests.
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