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The effect of addition bilimbi juice (*Averrhoa bilimbi* L.) with different concentrations and lengths of soaking time against decay of vannamei shrimp (*Litopenaeus vannamei*)

Rosmaidar[1*], Rastina[2], Nurliana[2], Hennivanda[1], Azhari[2], Putri Distyana Yolanda[3]

[1] Laboratory of Pharmacology, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh
[2]Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh
[3]Bachelor of Veterinary Medicine, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh
**Corresponding author: rosmaidarvet80@usk.ac.id*

Abstract

One of the fishery products that decomposes very rapidly is shrimp. Utilizing natural preservatives, such as *Averrhoa bilimbi* L. juice, is one method for maintaining the quality of food ingredients. This study aims to determine the effect of adding *A. bilimbi* L. juice with different concentrations and soaking times on vannamei shrimp decay. In this study, an experimental laboratory was used to examine decay using the Postma and H_2S tests. As much as 500 g of vannamei shrimp were divided into four treatment groups and two repetitions, with 5 g of each sample soaked in *A. bilimbi* L. juice with concentrations of 0%, 2.5%, 5%, 7.5%, and 10% for 0, 3, 6, and 9 hours. Early decay was observed at 0 and 3 hours after soaking vannamei shrimp in solutions with concentrations of 0%, 2.5%, 5%, 7.5%, and 10%. At concentrations of 5%, 7.5%, and 10%, there was no initial decay after soaking vannamei shrimp for six hours at a concentration of 0% and 2.5%. During 9 hours of soaking at concentrations of 0%, 2.5%, and 5%, initial decay occurred, whereas there was no initial decay at concentrations of 7.5% and 10%. The conclusion of this research is *A. bilimbi* L. juice with concentrations of 7.5% and 10% for 6 and 9 hours inhibits the decomposition of vannamei shrimp.

Keywords: Averrhoa bilimbi L., shrimp*,* decay*,* preservation

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Background

Shrimp are aquatic animals that live in seas, lakes, rivers, and ponds. Shrimp are divided into several types, including brackish water, seawater, and freshwater shrimp (Aidah, 2020). The nutrients in shrimp are usually used as a source of food ingredients needed, but shrimp can be contaminated by microorganisms, resulting in losses. This causes food to become unfit for consumption because of physical and chemical changes that cause food spoilage (Fauzan et al., 2021).

Shrimp spoilage is closely related to melanosis and microbial decay (Gokoglu & Yerlikaya, 2008). The formation of melanosis is a discoloration that occurs due to an enzymatic reaction by the polyphenol oxidase enzyme. The formation of melanosis can affect color parameters and affect consumer acceptance (Haard & Simpson, 2000). Melanosis is a decay process in shrimp during handling and storage (Perceka et al., 2014). Pacquit et al., (2007) stated that shrimp would begin to decompose after 10 hours of storage.

One of the microbes that play a role in the decomposition process is bacteria. According to Liviawaty and Eddy (2010), bacteria are the main microbes that cause the decomposition process in fresh shrimp. Bacteria can come from contaminated shrimp bodies from their habitat, processing environment, and storage areas. The number of organisms in the decomposition process is increased by physical damage, oxidation, color changes, pH changes, and odor changes (Ercolini, 2006). Another factor causing decay is a temperature which can regulate bacterial growth because the higher the temperature, the greater the growth rate (Ramli, 2001). This results in the need for prevention to maintain the quality and durability of food ingredients. One way that can be used to maintain quality is by using preservatives such as using bilimbi juice.

Fresh food can be preserved in various ways, such as salting, smoking, drying, and using natural preservatives. One example of natural preservatives is the use of bilimbi juice which can be used to maintain the quality of food ingredients.

Belimbing wuluh (bilimbi), or vegetable bilimbi, is a fruit that has a sour taste and is often used as a preservative for fish, food, and vegetable seasoning. Bilimbi is a plant that contains acid and functions as an antimicrobial to inhibit microbial growth directly. The acidic substances contained can disrupt the metabolism of bacteria. The disruption of this metabolism is due to the exchange of acidic ions (H^+) from the environment with the body's bacteria (Pakaya et al., 2014).

The various kinds of properties of bilimbi are because this fruit contains compounds including saponins, flavonoids, and polyphenols. Saponins are glycosides of sugars that bind to aglycones (Fahrunnida & Pratiwi, 2015). Flavonoids are a class of active compounds contained in the extract of the bilimbi, which has the potential to be anti-bacterial. So, bilimbi is often used as a preservative (Hamdanah et al., 2015).

Several studies on the use of bilimbi have been carried out, especially in the use of bilimbi leaves, but the use of bilimbi feelings still needs to be done. Based on the description of the background above, it is necessary to research the effect of giving bilimbi juice in inhibiting the decay of vannamei shrimp.

Materials and Method

Experimental Design

The method used in this study was the Postma test and the H_2S test with four treatments and two repetitions. The treatment was bilimbi juice (*Averrhoa bilimbi* L.) with concentrations of 2.5%, 5%, 7.5%, and 10% (Rastina et al., 2022) and vannamei shrimp weight of 5 g with long soaking times of 0, 3, 6, and 9 hours.

Sampling of Vannamei Shrimp

As much as 500 g of vannamei shrimp (*L. vannamei*) were taken from the Rukoh market, Banda Aceh. Sampling was carried out at 16.00 WIB using shrimp that had experienced a decline in quality, marked by physical changes in the shrimp, such as heads that started to turn red and skin that was wet and slimy. Then, the vannamei shrimp was brought to the Laboratory of Veterinary Public Health, Faculty of Veterinary Medicine, Universitas Syiah Kuala, Banda Aceh, to be cleaned using running water. Furthermore, each vannamei shrimp was put into a sterile plastic for a different treatment.

The Soaking Time of Vannamei Shrimp

This stage is the process of soaking vannamei shrimp (*L. vannamei*) with the addition of bilimbi (*A. bilimbi* L.) juice with different concentrations for each treatment, namely: P0= 5 g of vannamei shrimp + 0% belimbing wuluh; P1= 5 g of vannamei shrimp + 2.5% bilimbi; P2= 5 g of vannamei shrimp + 5% belimbing wuluh; P3= 5 g of vannamei shrimp + 7.5% bilimbi; P4= 5 g of vannamei shrimp + 10% bilimbi.

The amount of 5 g of soaked vannamei shrimp mixed with 100 ml of distilled water as a control for 2.5% treatment by mixing 2.5 ml of bilimbi juice with 97.5 ml of distilled water, for 5% treatment by mixing 5 ml of bilimbi juice with 95 ml of aquadest, for the 7.5% treatment by mixing 7.5 ml of bilimbi juice with 92.5 ml of distilled water and for the 10% treatment by mixing 10 ml of bilimbi juice with 90 ml of distilled water.

Postma Test

The Postma test for each treatment was aimed to observe the decomposition process. The extract of vannamei shrimp was made by taken one part of the shrimp sample with 10 parts of distilled water and put into a sterile plastic bag. Then it was homogenized in a stomacher for 10 minutes and filtered. The amount of 100 mg of MgO was put into a Petri dish with litmus paper. Furthermore, the Petri dish was placed in a water bath with a temperature of 50 °C for 5 minutes, then removed and observed for changes in the color of the litmus paper (Rahmi et al., 2022). A positive result is obtained if the litmus paper turns light blue, whereas if the litmus paper partially changes color, then the result is dubious, and if the litmus paper does not change color, then the result is negative.

H_2S test

The H_2S test for each treatment given also observed the decomposition process. Shrimp samples were taken and put into a Petri dish. Then the Petri dish is covered with filter paper, and one drop of 10% Pb acetate is dropped in the middle of the filter paper and avoid getting on the shrimp, then observe the changes that occur (Rahmi et al., 2022). In the H_2S test, a positive result is obtained if there is a brownish-black color around the Pb acetate drop, if there is no brownish-black color around the Pb acetate drop, then the result is negative.

Results and Discussion

The results of this research obtained from initial observations of decay based on the Postma test and the H_2S test on vannamei shrimp added with bilimbi juice can be seen in the Table 1.

Table 1. The results of Postma and H_2S tests

Repetition	Concentration /Treatment	Soaking time (hours)			
		0	3	6	9
$P_{1,2}$	P0	+	+	+	+
$P_{1,2}$	2.5%/P1	+	+	+	+
$P_{1,2}$	5%/P2	+	+	-	+
$P_{1,2}$	7.5%/P3	+	+	-	-
$P_{1,2}$	10%/P4	+	+	-	-

P0: control; P1,2,3,4: main treatment; (-): does not initiate early decay; (+): there was the start of decay; $P_{1,2}$: repetition.

Vannamei shrimp soaked with bilimbi juice concentrations of 0%, 2.5%, 5%, 7.5%, and 10% for 0 hours and 3 hours the results were positive marked by the change of red litmus paper to blue in the postma test, and there are brownish black spots around the drops of pb acetate on the filter paper in the H_2S test. This is because the shrimp used have experienced early decay, so the bacteria in the shrimp ferment and produce ammonia (NH_3) and the release of H_2S by the shrimp.

According to Marsidah (2017), NH_3 gas is produced by decomposed meat. Free NH_3 will then bind to the MgO reagent and form NH_3OH. Fresh meat does not produce NH_3OH because there is no free NH_3. In the Postma test, heating MgO can release NH_3, causing the colour of the red litmus paper to turn blue (Safrijal et al., 2017). The change of red litmus paper to blue indicates that the condition of the vannamei shrimp sample tends to be alkaline.

The H_2S test was conducted to detect the H_2S released by microorganisms in the sample. Decomposing bacteria that can produce H_2S, namely *Pseudomonas*. H_2S released by spoilage bacteria will bind with Pb acetate to become Pb Sulfite ($PbSO_3$). *Pseudomonas* bacteria also produce enzymes that can break down the fat components and protein components of foodstuffs, causing a foul odour and causing mucus (Dengen, 2015).

Soaking for 9 hours with concentrations of 0%, 2.5%, and 5% was positive, whereas concentrations of 7.5% and 10% showed negative results. This is because giving bilimbi juice can inhibit the decay of vannamei shrimp because of its citric acid content. According to Wiradimadja et al., (2015) citric acid is the highest acid content in bilimbi.

Vaname shrimp is a food ingredient preferred by the wider community because it is easy to obtain and has high economic value and nutritional content. The community, of course, tends to choose food ingredients that are still fresh, but if there is no good treatment of food, especially in the fisheries sector, it will easily experience a decrease in quality. This will provide opportunities for the growth of spoilage microbes and cause a decrease in quality and shelf life.

According to Jay (1986) that reported the signs of decay normally are wet and slimy skin surfaces. Shrimp rot can also be caused by blood vessels and dirt on the head of the shrimp. Shrimp undergo autolysis of protein and fat very quickly, and black spots appear when handled at high temperatures (Suparno, 2012).

Carambola fruit is classified as a source of organic acids, which contain several types of acids, such as acetic acid, citric acid and formic acid (Datu et al., 2015). According to Dibner and Buttin (2002) explained that almost all types of acids have strong antibacterial properties in inhibiting microbes. The highest acid content in bilimbi is citric acid (Wiradimatja et al., 2019). The mechanism of action of citric acid is to damage the bacterial cell wall and enter into the bacterial cell, disrupt the process of cell respiration, inhibit the activity of bacterial enzymes and suppress the translation of certain regulatory gene products (Ramadhinta et al., 2016).

The results of the 7.5% and 10% concentrations of the bilimbi juice were negative at 6 hours and 9 hours, indicating the bilimbi can maintain the quality of food ingredients. Prayogo et al., (2011) stated that giving bilimbi juice has been proven to be able to inhibit bacterial growth. The active compounds of flavonoids in bilimbi juice can form complexes with bacterial cell proteins through hydrogen bonds. The structure of the bacterial cell wall and cytoplasmic membrane, which contains protein, becomes unstable because the protein structure of the bacterial cell becomes damaged due to hydrogen bonds with flavonoids so that the protein of the bacterial cell loses its biological activity, as a result, the permeability function of the bacterial cell is disrupted, and the bacterial cell will experience lysis which results in the death of the bacterial cell.

Conclusion

Based on the results of this study, it concluded that the addition of bilimbi (*Averrhoa bilimbi* L.) juice with a concentration of 7.5% and 10% were inhibited the decay of vannamei shrimp with soaking times of 6 and 9 hours.

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