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Effect of chitosan of milkfish scale waste (*Chanos chanos*) on the preservation of tomato fruit (*Lycopersium esculentum*)

Intan Harlia, Fatmah Dhafir and Astija *

Biology Education Study Program, Faculty of Teaching Training and Education, Universitas Tadulako, Indonesia

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Abstract

Tomatoes (*Lycopersium esculentum*) provide excellent nutritional value for human health. However, their high water content and classification as climacteric fruits, which undergo quick ripening through respiration, make them vulnerable to harm during the post-harvest period. One method employed to prolong the longevity of tomatoes involves the application of a coating made from chitosan derived from milkfish scales. The objective of this study is to assess the impact of chitosan derived from milkfish scales (*Chanos chanos*) on the longevity of tomatoes by examining alterations in color, weight loss, pH level (acidity), and vitamin C content. This study employs a complete random design (CRD) experiment consisting of five treatments: $P0 = 0\%$, $P1 = 1\%$, $P2 = 1.5\%$, $P3 = 2\%$, and $P4 = 2.5\%$. Each treatment is replicated three times. The metrics recorded encompassed the tomato's color, weight loss measured by comparing the initial weight with subsequent weights, pH value (indicating acidity), and monitoring of vitamin C levels.

Keywords: *Chanos chanos;* Chitosan; *Lycopersium esculentum*; Milkfish; Tomato

1. Introduction

Tomatoes, formally referred to as *Lycopersicum esculentum*, are rich in a diverse range of nutrients, including vitamins A, C, and E, flavonoids, fiber, carotenoids, lycopene, and several minerals. These nutrients exert beneficial impacts on human health. Because tomatoes have a high water content, their harvesting leaves them vulnerable to damage. Tomatoes typically contain a water content ranging from 93% to 95% [8]. Furthermore, [5] classify tomatoes as climacteric fruits due to their tendency to experience a rapid surge in respiration and ethylene production throughout the ripening process. The respiration patterns will affect the tomatoes quality. Increasing the pace at which a person breathes causes a significant change in the factors that affect weight loss, the color of tomatoes, texture deterioration, and nutritional content [19].

At present, we utilize a range of methods to address the issues that arise after harvesting tomatoes, including storing them in a controlled atmosphere and packaging them with plastic materials. Nevertheless, these methods are susceptible to several limitations. Operating in a controlled environment necessitates expensive equipment, whereas using plastic packaging to preserve fruit may result in the introduction of hazardous chemicals if the fruit directly touches the packaging material [17].

Like other food products, tomatoes can benefit from the application of an edible coating. The coating functions as a barrier, preventing the flow of moisture, oxygen, and volatile chemicals [7]. This coating process might be considered a solution for extending the shelf life and maintaining the quality of a product. We can develop and utilize chitosan, a potential fruit covering material, efficiently [4].

Corresponding author: Astija

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Chitin deacetylation is a chemical and enzymatic procedure that produces chitosan, a naturally occurring biopolymer in the polysaccharide category. Chitosan is the second most prevalent biopolymer, behind cellulose [11]. Chitosan acts as a barrier to reduce respiration rate, inhibit fungus development, delay fruit ripening, and decrease ethylene production. Research found that using chitosan as a food coating can significantly extend the shelf life of tomatoes [2]. Fish scales directly yield chitosan by extracting the chitin. The fishing industry haphazardly discards fish scales as solid trash.

Milkfish production in Central Sulawesi is on the rise. Significant milkfish production will result in increased trash generation, necessitating efficient management. Furthermore, milkfish scale debris possesses advantageous chitosan characteristics due to its lower ash or mineral content in comparison to the majority of marine fish [18]. This results in a significant degree of chitosan purity and optimal chitosan functionality [10]

The objective of this work is to harness the potential of chitosan as a preservative by utilizing milkfish scales as a source material for chitosan production. Next, we will use chitosan as a consumable coating to protect tomatoes.

2. Approach

The goal of this experimental investigation is to determine the impact of treating the chitosan layer of milkfish scales (*Chanos chanos*) on tomato preservation. This study used a complete random design (CRD) with five treatment levels: $P0 = 0\%$, P1 = 1%, P2 = 1.5%, P3 = 2%, and P4 = 2.5%, each repeated four times. We assessed the tomatoes based on their weight loss, pH level, acidity, and vitamin C content.

2.1. The sample preparation process

We acquired milkfish-scale trash samples (*Chanos chanos*) from milkfish vendors in the Palu City Traditional Market. We cleanse the acquired fish scales and then expose them to direct sunlight for 1-2 days, or until they achieve full desiccation. After the fish scales have dried, we pulverize them until they have a smooth texture, which makes it easier to turn them into chitosan.

2.2. Chitosan production

Chitosan is produced by extracting chitin from milkfish scales, which weigh up to 2000 grams. We then deproteinize the chitin using a 3.5% NaOH solution at a ratio of 1:10 (grams of scales per milliliter of NaOH) and at a temperature of 65 \degree C–70 \degree C. This procedure vields a solid substance, which we then rinse with water until the pH reaches a neutral level. We then set an oven at 50 \circ C to dehydrate the extracted substance. The sample then undergoes demineralization extraction using a 1 N HCl solution at a ratio of 1:6 (grams of sample per milliliter of HCl), stirring it for 30 minutes at a temperature of 30 °C. This process produces a precipitate, followed by a neutral pH wash and drying at 50 °C. The resulting product is known as chitin. We conduct a color test to confirm the presence of chitin compounds in the sample. This involves reacting 0.1 gram of the sample with a drop of lugol and iodine solution. The lugol droplet will cause the sample to change color to brown, which will then further change to violet upon the addition of droplets of sulfuric acid solution.

Furthermore, we subjected the previously demineralized chitin to a deacetylation procedure using 40% NaOH at a ratio of 1:10 (grams of chitin to milliliters of NaOH) at a temperature of 90 degrees Celsius for 90 minutes, which resulted in the formation of a precipitate. We rinsed the object with water until the pH reached a neutral level, and then dried it at a temperature of 60 degrees Celsius. Subsequently, we subjected the acquired data to a color test procedure in order to ascertain the existence of chitosan content in the sample. The procedure entails measuring 0.1 grams of the sample and placing it in a container. Next, apply a chitosan solution containing ninhydrine to the sample and let it sit for five minutes.

2.3. Edibels Coating Manufacturing

Next, we convert the chitosan into a consumable layer by dissolving it in 10% formic acid. We chose formic acid as the solvent because it has the ideal solubility for chitosan at this concentration [6]. Subsequently, we utilize the resulting solution as a consumable coating for tomatoes, submerging them in it for around one minute.

We preserve tomatoes with a protective coating for a period of 9 days, during which we observe changes in color, weight loss, acidity level (pH value), and vitamin C levels at 3 DAT (Days After Treatment) 6 DAT (Days After Treatment) and 9 DAT (Days After Treatment).

3. Results and discussion

3.1. Tomato Fruit Discoloration

We can visually see the change in color of tomato fruit. The color of the tomato is a criterion for assessing its ripeness. Figure 1 illustrates the observed variations in tomato fruit color at the stadium level.

Figure 1 Color Stadium Change

*Information: 0 = *Turning* 1 = *Pink/Light Orange* 2 = *Light Red*) 3 = *Red*

The observational data showed that the P0 treatment, which was used as the control, had the most significant change in the phases of tomato coloring. In contrast, the P4 treatment, with a chitosan layer concentration of 2.5%, effectively prevented the advancement of tomato color change phases. Before the identification of nine days after treatment (DAT) in treated tomatoes, some of them were still undergoing the ripening process.

The empirical evidence suggests that tomatoes consistently exhibit a gradual increase in discoloration across all treatments, ranging from 3 DAT to 9 DAT. The change in color of tomatoes occurs because the chlorophyll, which is found in unripe tomatoes, is converted into natural pigments like yellow and red as they grow, depending on the specific type of tomato. The fruit loses its green hue due to the conversion of chloroplasts into chromoplasts, which house carotenoid pigments.

The control treatment, referred to as P0, showed the most significant changes in the color of tomato fruit. [14] and [12] have claimed that an increased respiratory rate can accelerate the degradation of chlorophyll and the production of pigments, hence speeding up the color change process. The application of a chitosan coating on milkfish scales demonstrates the ability of the chitosan layer to slow down the color change process. This is particularly evident in the P4 treatment, which has a chitosan layer concentration of 2.5%. This therapy effectively inhibits the stages of tomato discoloration. While tomatoes ini 9 DAT have been seen, there are still others that have not yet reached the stage of ripening. The application of a coating can effectively safeguard chlorophyll from degradation by obstructing the exchange of O2 and CO2 gases, to which the fruit exhibits high resistance [12]. This hinders the transformation of chlorophyll into carotene, hence preserving the color of the tomato.

3.2. Tomato's Effect on Weight Loss

The reduction in mass of fruit is a consequence of the metabolic process known as respiration. The physiological process of respiration in fruit involves the absorption of oxygen, the oxidation of organic molecules to produce energy, and the subsequent release of carbon dioxide and water vapor, leading to a decrease in weight [12].

Figure 2, displays the results obtained from the observations. The tomatoes experience an increased trend in shrinkage during storage, with an observed rise from 3 DAT to 9 DAT. Weight loss is more probable when fruits mature and are preserved for extended durations [13]. The increase in weight is a consequence of the transpiration process, wherein water is released from tomatoes into the environment, resulting in a decrease in the water content of tomatoes and causing them to reduce in weight.

Among all the treatments, the P2 treatment, which had a chitosan layer concentration of 1.5%, had the most effectiveness in minimizing weight loss in tomatoes throughout of 9 DAT storage. It outperformed all other treatments in terms of the percentage of weight loss. The chitosan edible layer functions as a barrier, resulting in a slowdown of the respiration process and impacting the rate of weight loss. The implementation of an edible coating might diminish or potentially obstruct the uptake of oxygen in tomatoes, so impeding the respiratory process dependent on oxygen, reducing the probability of water loss, and averting loss of weight [16]. The findings are consistent with the results which clarifies that the chitosan edible layer can slow down the rate of weight loss because of its capacity to form a coating [15]. This coating has the capacity to impede breathing, leading to a decrease in weight loss.

Based on the observations, it was found that the P4 treatment, which had a chitosan layer content of 2.5%, led to the highest level of weight reduction. This medication consistently shown a substantial improvement in weight reduction across all observations. It is anticipated that the application of this treatment resulted in an excessive deposition of chitosan, successfully sealing nearly all of the pores on the tomato. The process of anaerobic respiration in tomatoes results in their decomposition, leading to rotting. This technique expedites the maturation of tomatoes and leads to a gradual reduction in their mass. A high concentration of the edible layer causes it to become too thick, which impairs its usage and leads to anaerobic respiration. Almost all tomato stomata are blocked, it leads to anaerobic respiration, which in turn generates carbon dioxide (CO2) [7]. This phenomenon arises when the respiration process is impeded due to the fruit pores being covered by a thick layer of chitosan. During anaerobic respiration, cells experience a metabolic process that causes the fruit to deteriorate rapidly. As a consequence, there is an elevated rate of weight loss and the tomato undergoes spoilage.

Figure 2 Weight Loss Of Tomato Fruit

3.3. pH value (acidity)

The pH of a fruit is determined by the concentration of organic acids it contains. A rise in pH indicates a reduction in the acidity level of the fruit. A low pH value indicates that the organic acids in tomatoes are still intact and in a healthy state. During storage, tomatoes undergo a process of acid reduction or pH increase. The rise in pH is a result of reduced acid production occurring during storage [1]

Figure 3 pH Value of Tomato Fruit

The pH value of tomato acidity often increases significantly over the 9 DAT, as shown in Figure 3. The pH value of fruit increases during storage due to the presence of acid in the fruit tissue [14]. If the titrated acid content drops during storage, the pH value of the tomato fruit will increase, resulting in a higher level of ripeness.

The observation findings indicated that treating tomatoes with a chitosan layer derived from milkfish scales at a concentration of 1.5% (P1) resulted in a significant rise in the average pH value compared to the treatment without a chitosan layer (P0, control). The treatment of the chitosan layer with a concentration of up to 1.5% is believed to have the capacity to inhibit the pace at which the pH value increases, hence impacting the ripening process of tomatoes. According to [1], ripe tomatoes often have a higher pH value than other tomatoes. Therefore, using a coating treatment can help reduce the rate at which the pH value increases, compared to not using a coating treatment. Unlike the control treatment, the treatment with the P0 layer exhibits a significant rise in pH value. The rise in the pH level of tomatoes after storage is a result of the reduction in the concentration of organic acids in tomatoes [15].

3.4. Observation of Vitamin C Levels

Vitamin C, also known as ascorbic acid, is a water-soluble vitamin. It has a white crystalline structure and is categorized as an organic acid. It has a sour taste but does not have a smell. Vitamin C is very susceptible to degradation compared to other vitamin groups. Vitamin C has high solubility in water and is susceptible to oxidation, which is further facilitated by factors such as heat, radiation, alkali, enzymes, oxidizers, as well as copper and iron catalysts [9].

Tomatoes are considered an excellent source of vitamin C, since 100 grams of tomatoes can provide 20% or more of the recommended daily intake of vitamin C. Fresh tomatoes provide 40 mg of vitamin C per 100 g of fruit [3]. Tomatoes are rich in vitamin C, however the vitamin C content gradually diminishes as tomatoes mature.

Figure 4 displays the results of observing the effects of different milkfish scale chitosan coatings on storage times ranging from 3 DAT to 9 DAT. Each observation generally shows a decline in the vitamin C levels in tomato juice. Typically, the concentration of vitamin C in tomatoes will drop as they mature. Throughout the process of maturation, there is a decline in the concentration of organic acids. The reduction in organic acids is a result of the respiration process that converts organic acids into sugars [12].

Observational results indicated that the P3 treatment, with a chitosan layer content of 2.5%, exhibited a minimal reduction in vitamin C levels in comparison to the uncoated treatment. According to [12], treating the chitosan layer with a 2.5% concentration is the most effective in preventing the diffusion of O2 into fruit tissue and slowing down the oxidation reaction that leads to vitamin C degradation. Conversely, in tomatoes that did not get a chitosan P0 (control) layer treatment, there was a significant drop in vitamin C levels seen. Tomatoes without milkfish scales experience a hindered passage of oxygen into their tissues, leading to continuous breakdown of vitamin C.

Figure 4. Vitamin C of Tomato Fruit

4. Conclusion

Using milkfish scale chitosan as an edible coating material can preserve tomatoes during storage by preventing changes in color, weight loss, pH increase, and vitamin C decrease. The most effective concentration for preserving tomatoes with a layer of milkfish scale chitosan for a storage period of nine days was found to be 2.5% for preventing color change and reducing vitamin C levels, and 1.5% for minimizing weight loss and controlling the increase in pH value (degree of acidity).

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

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