# Effect of Hypoiodous Acis (HIO) Treatment on pH and Discoloration of Snake Fruit (Salacca zalacca) during Room Temperature Storage

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# EFFECT OF HYPOIODOUS ACID (HIO) TREATMENT ON PH AND DISCOLORATION OF SNAKE FRUIT (*Salacca zalacca*) DURING ROOM TEMPERATURE STORAGE

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## ABSTRACT

Snake fruit (*Salacca zalacca*) is a tropical fruit with short shelf time because it is highly susceptible to fungal infection and enzymatic browning reaction. The discoloration of the fruit caused by enzymatic browning is one of the fruit damage that causes activation of polyphenol oxidase enzyme (PPO). One of possible alternative to inhibitory agents, which is known as an antibacterial and antifungal compound. The purpose of this study was to analyze correlation between discoloration and pH value for treatment of fruit after HIO during 15 days at room temprature storage (30 ± 5 ◦ C). Discoloration test refers to L *, a * and b * value was done by using digital color meter (Apple, USA) and pH value test was done by using pH meter (Hanna, USA). The correlation between discoloration of snake fruit with HIO treatment refers to L *, a * and b * value and pH.

**Keywords:** discoloration , hypoiodous acid, pH , snake fruits.

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## INTRODUCTION

Snake fruit is one of the original tropical fruits of Indonesia. Just like fruit in general, snake fruit is a perishable commodity. One indication of damage to fruit is the occurrence of color changes (Buve et al., 2018). This is caused by changes in the color of the fruit will reduce the quality of taste, improve alkaline properties and reduce nutrition (Cortez-Vega et al., 2008). Color changes in the fruit itself can be through non-enzymatic or enzymatic phenomena. This enzymatic browning occurs due to the oxidation of phenolic compounds in the fruit which is catalyzed by the enzyme polyphenol oscidase (PPO) when the fruit is physically damaged and will then produce quinone compounds which cause the color to brown (Gomes et al., 2014)

The activity of PPO enzymes that are responsible for color changes in fruit can actually be inhibited either directly or indirectly with various compounds (Mesquita and Queiroz, 2013). Ascorbic acid, citric acid and potassium bisulfite are some compounds that have been used to inhibit PPO enzyme activity in fruit . As another example, the use of NaCl solution (Iannou and Ghoul, 2013) but this method leaves a negative impact in the form of taste due to treatment. One alternative that can be used is to use Hypoiodous acid (HIO). HIO itself is a weak

acid compound known as antibacterial and antifungal (Bafort et al., 2014). HIO is a compound formed from the reaction of two substrates, namely $H_2O_2$ and KI which are catalyzed by peroxidase enzymes (Kupper et al., 1998). The use of HIO solution in fruit, especially in order to inhibit color changes in snake fruit due to enzymatic reactions to date has never been done.

The purpose of this study was to analyze the correlation between the color changes shown by the values of L *, a * and b * with the pH value of snake fruit preserved with HIO solution.

## MATERIALS AND METHODS

### Materials

#### Chemical materials and enzyme

$H_2O_2$ (0. 2 mM) , KI ( 0. 2 mM) were purchased from Roche (Germany). The snake fruits was obtained from local farm. Daikon radish for the source of peroxidase enzyme from modern market in Tembalang, Semarang, Indonesia. Aquadest and phosphate buffer were obtained from Integrated Laboratory Diponegoro University, Semarang, Indonesia.

### Methods

#### HIO solution preparation

HIO solution was made from $H_2O_2$ (0. 2 mM), KI ( 0. 2 mM), and peroxi dase enzyme in ratio 4.5: 4. 5: 1. All of the solutions were mixed in beaker glass and stirred. The mixture was allowed to react for 6 minutes. After 6 minutes, the HIO solution is ready to use.

#### Peroxidase enzyme preparation

Preparation of peroxi in enzyme from Daikon radi sh following the method from Lintjewas *et al*. (2015) to obta in the crude extract of peroxi dase enzyme from natural sources. Radish was washed and cut into small pieces. The cuts were weighed and blended with phosphate buffer (0.01 M, pH 7) in a ratio of 1 : 4. The blended radish was filtered with cloth filter to obtain the juice. The juice is centrifuged for 10 minutes with the speed of 1700 rpm using Scilogex DM0412 centrifuge. The supernatant and s ediments were separated by filter cloth and the supernatant as used as peroxi dase enzyme.

#### Fruit juices preparation

Preperation of snake fruit w a s following the method from Gorinstein *et al.* (2010) with modification. The flesh of snake fruit was cutted without using steel knives. The peeled fruits then weighed and blended. The snake fruits juices then filtered with filter paper and the juices were stored in tube and then treated and further analyzed. The snake fruit juices was used for color analysis. During the treatment and analysis process, the sample temperature is maintained as not to exceed 15 ° C.

**Color analysis**

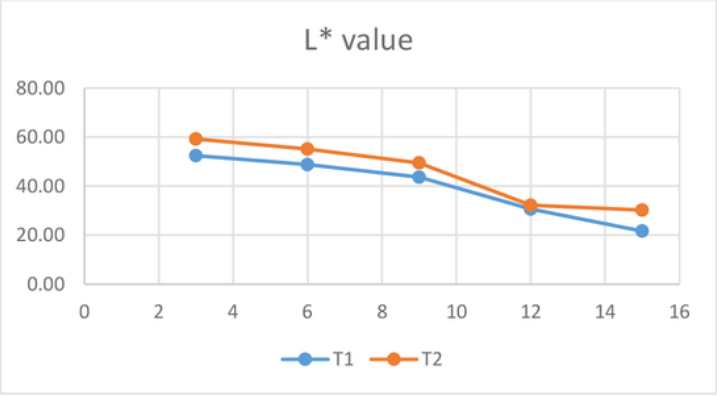
Color change testing is done by digital color meter application (Apple, USA) on Machintos. The analysis performed is the value of L *, a * and b *. Samples that have been treated are placed under a camera connected to a computer. Furthermore, measurements are directed at the sample display tested on the display screen and the measurement results of L *, a * and b * values will appear on the computer screen.

**pH analysis**

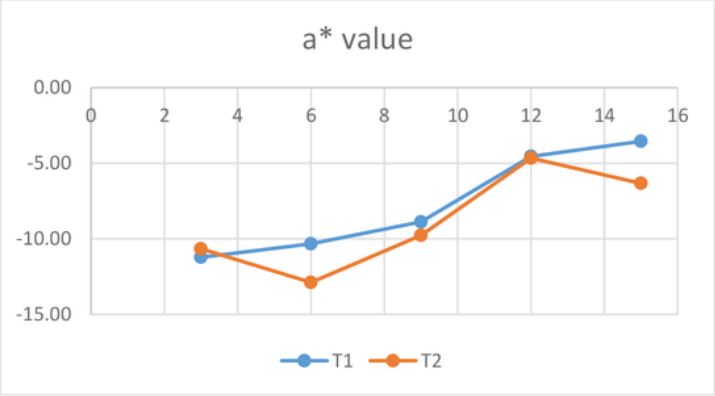
PH testing is done by preparing 5 ml of snake fruit juice for further testing using a pH meter (Hanna, USA). Tool calibration was carried out using buffer solutions 4 and 7. pH measurements were carried out by dipping the pH meter electrode into the sample (AOAC, 1995).

## RESULTS AND DISCUSSION

**Discoloration**




a)

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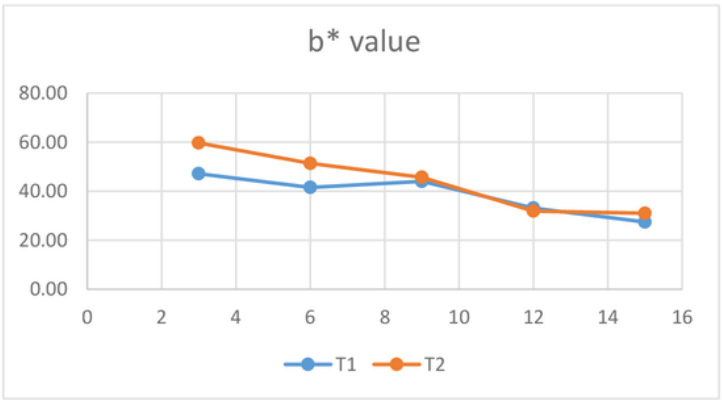



c )

Fig. 1 The color changes of snake fruit with 0.2 mM of HIO and 0 of HIO ; a) L value of snake fruit with 0.2 mM of HIO and 0 of HIO ; b) a value snake fruit with 0.2 mM of HIO and 0 of HIO c) b value snake fruit with 0.2 mM of HIO and 0 of HIO

Color changes in food ingredients (including fruit) can be known based on the values of L *, a * and b *. The value L * denotes the brightness level of a food ingredient, the value of a * denotes the reddish color while the b * represents the greenish color. In Fig. 1, it can be seen that the browning reaction on snake fruit which is treated with HIO can be inhibited. This is indicated by a slower decrease in L * value in snake fruit with HIO treatment which means PPO enzyme activity can be suppressed by HIO because HIO itself can bind to the allosteric side of PPO enzyme which plays a role in producing quinone compounds that cause browning. The allosteric side of the enzyme itself is a part that is able to bind to the substrate but is not the active side of the enzyme (Purich, 2010). So that it can be said that HIO is an inhibitor because it can inhibit the activity rate of PPO enzymes. HIO itself is also an acid which has a function as an oxidizer (Alkorta et al., 2008). Then in terms of a * value it can be seen that the higher the value of a* indicates the intensity of the high reddish color and vice versa. The results showed that snake fruit with HIO treatment had a lower a * value than snake fruit which was not treated with HIO. The value of a* itself is not a direct indicator that influences browning reactions because the values of a* and b* themselves have a weak relationship with PPO enzyme activity (Galvis-Sanchez, 2004). Whereas from the b* value, it can be seen that the snake fruit which was treated with HIO and snake fruit which was not given HIO treatment was not much different, but those who were not treated with HIO had a lower b* value The value of b * itself cannot be used as a direct indicator that affects browning reactions because the value of b * has a weak relationship with PPO enzyme activity (Rocha and Morais, 2001). So that based on the analysis of the L *, a * and b * values it can be concluded that the HIO compound can be used as a compound to inhibit browning reactions indicated by the L * value, this is because only the L * value has a correlation with the PPO enzyme activity responsible for browning reaction (Gomes et al., 2014).

**pH Value**

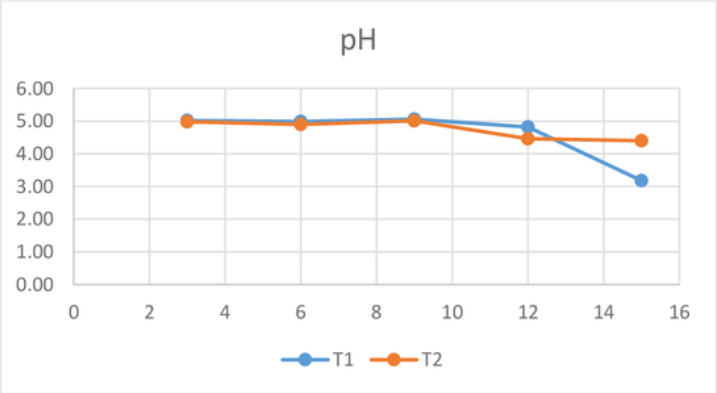
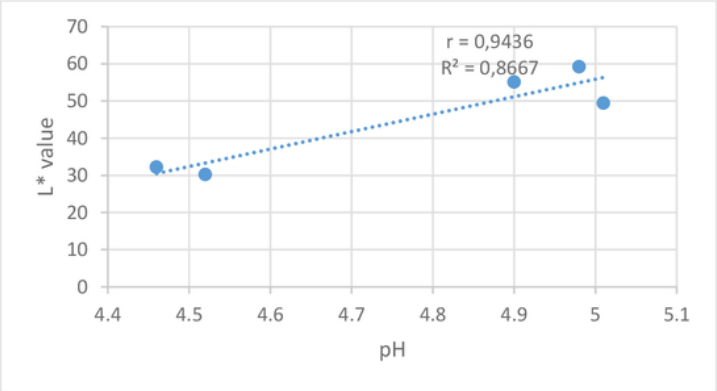


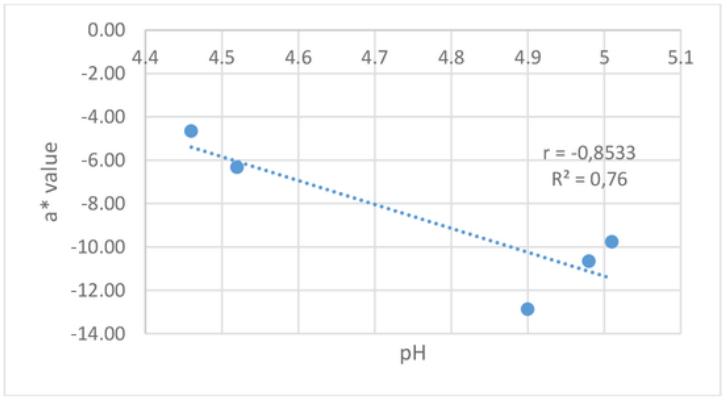

Fig. 2 The pH changes of snake fruit with 0.2 mM of HIO and 0 of HIO

Based on the graph, it is known that the bark which was treated with HIO had a more stable pH compared to the bark which was not treated with HIO. This shows that the HIO treatment on snake fruit allows to inhibit browning reactions seen from the ability to inhibit the decrease in pH value. Browning reaction on the fruit itself is a serious problem because it can reduce the quality of taste, improve alkaline properties and damage nutrients from the fruit itself (Cortez Vega et al., 2008).

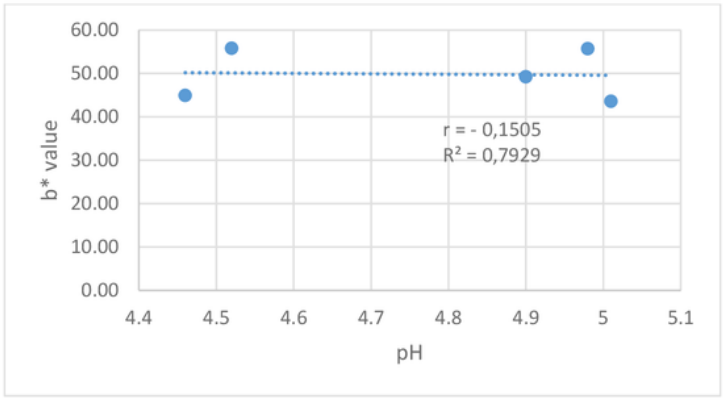
**Correlation between changes in color and pH**




a)




b )




c )

Fig. 3 The color changes of snake fruit with 0.2 mM of HIO and 0 of HIO ; a) Correlation between L value and pH of snake fruit with 0.2 mM of HIO and 0 of HIO ; b) Correlation between a value and pH snake fruit with 0.2 mM of HIO and 0 of HIO c) Correlation between b value and pH snake fruit with 0.2 mM of HIO and 0 of HIO

Based on the correlation calculation, the L * value has a high correlation with the pH value as indicated by the correlation value (r) of 0.9436 with a sign of the correlation value of 0.0159 . If thecorrelation significance value is p <0.05, this value is considered significant (Vidau et al., 2011). This positive correlation value indicates that the correlation between L * and pH is directly proportional. This means that when the L * value decreases, the pH will also decrease and vice versa. Meanwhile, the values of a * and b * have r values of (-) 0.8533 and (-) 0, 15 0 5, respectively, with the significance of each correlation and 0.0 659 and 0.8091 . This value indicates that there is no connection between changes in reddish and yellowish color with changes in pH values associated with browning reactions.This is because the values of a * and b * have an insignificant relationship with the activity of the PPO enzyme which is a catalyst for browning reaction (Zhang and Shao, 2015). Based on the correlation analysis, it can be

concluded that the change in color L * has a close relationship with the pH value, while the values of a * and b * are not.

## CONCLUSION

Based on the analysis of color changes through the values of L *, a * and b * and pH it can be concluded that the HIO treatment can inhibit browning reactions on snake fruit stored at room temperature. The color change shown with the L * value has a strong relationship with changes in pH values while the values of a * and b * are not because they are not directly related to the activity of the PPO enzyme which is responsible for browning reactions on the snake fruit itself.

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