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Abstract. Snake fruit (*Salacca edulis* Reinw.) is a tropical fruit with short shelf time because it is highly susceptible on fungal infection and enzymatic browning reaction. The color and pH changes of the fruit caused by enzymatic browning is one of the indicator of the damaged fruit that was caused by activation of polyphenol oxidase enzyme (PPO). One of the possible alternative agent to inhibit this reaction is using hypoiodous acid (HIO), which was known as an antibacterial and antifungal compound. The purpose of this study was to analyze correlation between color changes and pH value for HIO treatment on snake fruit during 15 days at room temperature storage (30±5^{o}C). Color changes analyze referred to L*a*b* analysis was done using digital color meter and the pH test was done by using portable pH meter. The result indicated that HIO could maintain color changes (L*a*b* value) and pH of snake fruit during storage at room temperature which caused by enzymatic browning reaction. As conclusion, HIO could be used as alternative compound to inhibit enzymatic browning in snake fruit through the detection in color and pH changes.

Keywords: hypoiodous acid, pH changes, snake fruit, temperature storage

1. Introduction

Snake fruit (*Salacca edulis* Reinw.) is one of the tropical fruit on Indonesia. Just like fruit in general, snake fruit is a perishable commodity. One indication of damage on fruit is the occurrence of color changes [1]. This phenomena reduces the quality of fruit, which is taste and nutrition [2]. Color changes in the fruit itself can be appeared through non enzymatic and enzymatic reaction. The enzymatic browning occurs due to the oxidation of phenolic compounds in the fruit which is catalyzed by the polyphenol oxidase enzyme (PPO) when the fruit has a physical damage and then will produce

quinone compound which caused a brown color [3]. The activity of PPO enzyme that are responsible for color changes in fruit can actually be inhibited directly or indirectly with various compounds [4]. Ascorbic acid, citric acid and potassium bisulfite are some compounds that have been used to inhibit PPO enzyme that caused enzymatic browning in fruit. The use of NaCl solution [5] as example, may inhibit the enzymatic activity but this method leave a negative aftertaste due to treatment. The use of *hypoiodous* acid (HIO) provides future method for hindering the enzymatic browning in fruit. HIO is a weak acid compound known as antibacterial and antifungal [6]. HIO is a compound that was generated form the reaction of two substrates, namely H_2O_2 and KI which are catalyzed by peroxidase enzyme [7]. Therefore, this research was done to use of HIO in fruit in order to inhibit the enzymatic browning through the appearance in color and pH changes in snake fruit. This study was also analysed the correlation between the color changes and the pH value in snake fruit preserved with HIO solution.

2. Material and Methods

2.1. Materials

2.1.1. Chemical materials and enzyme. H_2O_2 and KI were purchased from Roche (Germany) and 0.2 mM of those substrates was applied. The snake fruits was obtained from local farm. Horseradish for the source of peroxidase enzyme from modern market in Tembalang, Semarang, Indonesia. Aquadest and phosphate buffer were obtained from Center of Research and Services-Diponegoro University, Indonesia.

2.2. Methods

2.2.1. HIO solution preparation. The method from previous research [8] with modification was made as follows: H_2O_2 (0.2 mM), KI (0.2 mM), and peroxidase enzyme (4U) was mixed at ratio 4.5:4.5:1. All of the solutions were mixed in beaker glass then stirred for one minute. The mixture was allowed to react for 6 minutes. After 6 minutes of reaction time, the HIO solution is ready to use.

2.2.2. Peroxidase enzyme preparation. The daikon radish was used as the method of previous researcher [9] to obtain the crude extract of peroxidase enzyme. 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 filter cloth to obtain the high concentration of juice. The juice was then centrifuged for 10 minutes with the speed of 10000 rpm using Scilogex DM0412 centrifuge to obtain supernatant. Supernatant was then separated by Whatman filter and was used as peroxidase enzyme.

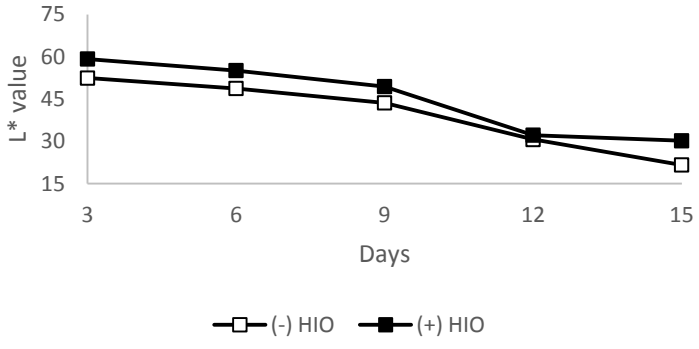
2.2.3. Fruit juices preparation, The method was initiated by cutting snake fruit into small pieces using sharp-plastic knives. The peeled fruits then weighed and blended. The snake fruits juices then filtered with Whatman filter and the juices were collected in tube and then treated for further analyzed. The snake fruit juices was used for color analysis. During the treatment and analysis process, the sample is maintained in 15±2°C and no more than 5 minute prior to use.

2.2.4. Color changes analyze. The method was done by the software of Digital Color Meter (Apple, USA) on Macintosh computer. The analysis was performed by determining value of L*a*b*. Samples was treated in the mini studio box under the iSight camera with the fixed lighting at 50 lumen. Furthermore, measurements were conducted by pointing samples from 7 points and the result of L*a*b* values would appear on the display screen.

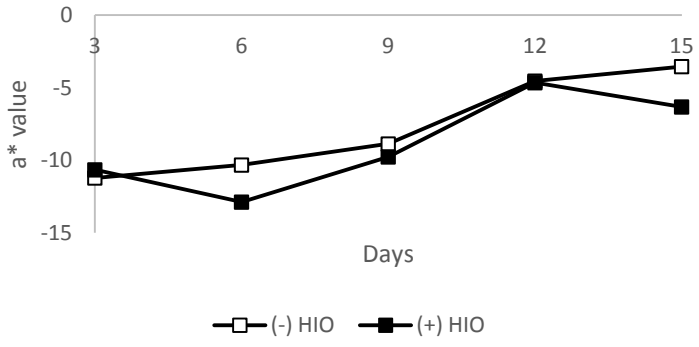
2.2.5. pH value analyze. The analysis was done by the portable pH meter (Isobe, Germany) and calibrated with buffer solution at pH 4.01 and 7.01 prior to use. The analysis was carried out by dipping the pH meter probe into the sample.

3. Result and Discussions

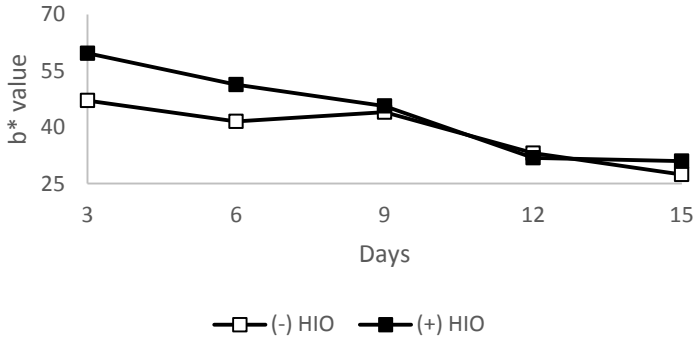
3.1. Color Changes Analysis



(a)



(b)



(c)

Figure 1. The color changes (L*a*b* value) of snake fruit treated with and without HIO solution. The value of L*a*b* represented at (a), (b), (c).

Color changes in food ingredients (including fruit) might be known from the values of L*a*b* as can be seen on Figure 1. The L* value denotes the brightness level of a fruit, the a* value denotes the reddish color while the b* value represents the greenish color. From Figure 1, it can be seen that the browning reaction on treated–snake fruit with HIO can be inhibited since the beginning of storage time as slower decrease in L* value which means the polyphenol oxidase enzyme (PPO) suppression by HIO. This may be appeared since HIO may bind to the allosteric side of PPO enzyme which plays

role in quinone production. The allosteric side of enzyme itself is a part that able to bind the substrate but not the active side of the enzyme [10]. So, HIO may decrease the activity rate of PPO enzyme. HIO is also an acid which has function as an oxidizer [11]. Then in terms of a* value, it can be seen that the higher value of a* indicates the intensity of reddish color. The results showed that snake fruit treated with HIO had lower a* value than those of untreated with HIO. The a* value may not addressed as indicator of browning reactions since a* value had less relationship with PPO enzyme activity [12]. Whereas b* value on treated and untreated HIO–sample were not much differently appear, but still the untreated HIO–sample had lower in b* value. Similar to a* value, b* value couldn't be used as a primary indicator of browning reaction since b* value had a weak relationship with PPO enzyme activity [13]. So based on the analysis of the L*a*b* values, it can be concluded that the HIO compound might be used as inhibitor in browning reaction based on the L* value. This result shows linear with other researcher data that L* value had strong correlation with the PPO enzyme which responsible for browning reaction [3].

3.2. pH Analysis

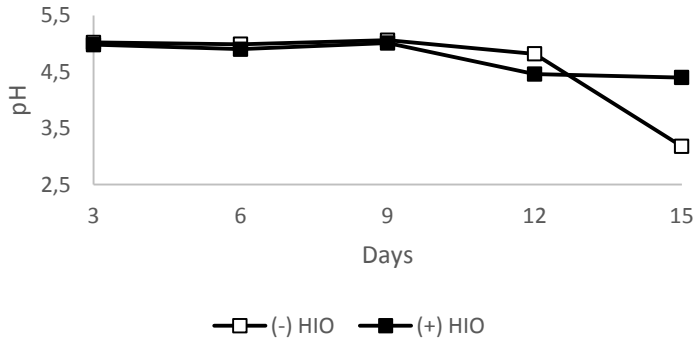


Figure 2. pH change in snake fruit treated and untreated with HIO solution

Based on the Figure 2, it is known that the snake fruit which was treated with HIO had more stable pH if compared to untreated snake fruit. The pH value of the fruit may primarily affect the taste of the fruit. The lower pH value means the higher acidity of the fruit and if the pH value higher means the acidity of the fruit will be lower [14]. The taste in the fruit was also influenced by the browning reaction. Browning reaction on the fruit results serious problem due to reduction in the quality of taste and reduce the pH of fruit resulting in the damage the nutrient in the fruit [2]. Based on this analysis, the HIO treatment on snake fruit allowed browning reaction to be inhibited as can be seen from the hindrance of pH reduction. The inhibition might be explained by the inhibition in metabolic reactions rate and microbiological damage which causes the change in pH value. This is linear with the founding that HIO have long been known as compound that might inhibit bacterial and microbial growth which that caused microbiological damage [8].

4. Conclusion

Based on this study, the snake fruit which is treated with HIO had higher value in L* and provided the more stable in pH value compare non-treated snake fruits with HIO solution. Thus, HIO solution performed browning reaction inhibition compound in snake fruit in order to maintain the quality of the snake fruit.

5. References

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