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Rate of physical appearance changes on yellowness in *salak* during preservation in room storage

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ABSTRACT

Background*: Discoloration was one indicator of food damage including in fruits, since the change may be used as a quality measurement. Salak became one of the commodities that often experience the browning reaction that may change the color. Since the yellow was close to the consumer preference along the preservation in salak, this color should be notified and may be represented as polyphenol change.*
Objectives*: The focus of this study was to determine the pattern of inhibition of browning reactions in salak using HIO.*
Methods*: This research determined the color level of yellow using digital color meter of salak that was stored in room temperature in aseptic treatment for 12 days.*
Result*: Discoloration appeared in salak and it was clearly determined the change since a week of storage. The rate of discoloration was able to be detected highly after one week of storage.*
Conclusion*: The discoloration of yellow color and the rate of salak could be detected specifically based on the day of storage. This research may open the information for the consumer to predict the storage time of salak based on the appearance of yellow color.*

Keyword*: physical appearance; yellow color; salak; browning; storage*

INTRODUCTION

Fruit is one of the commodities that is easily damaged. One of the damage to the fruit is browning. Enzymatic browning of the fruit is divided into two namely enzymatic and non-enzymatic browning[1]. The enzymatic browning reaction in the fruit can be caused by the oxidative reaction of polyphenol oxidase (PPO) enzyme which reacts with oxygen which will produce a brownish color in the fruit[2]. Many fruits of tropical or subtropical origin can run to browning [3] like *salak*.

Salak cultivar Pondoh is one of infamous native tropical fruits in Sleman, Yogyakarta. This fruit is favored not only by local consumers but also overseas consumers for its taste and crunchy texture. Besides, it is also rich in nutrient content including dietary fiber and antioxidant[4]. According to data from National Statistic Agency, it was recorded that export of *salak* in 2015 and 2016 had increased from 758 to 790 ton followed by increasing demand along with its growing popularity. Some of the destination countries for the export are Singapore, Middle East country, Netherlands, Hongkong, and China[5]. However, *salak* is perishable commodities and have short-aging. In general, *salak* can be stored at ±7 days at room temperature and it will be prolonged at low temperature[6].

The process of color change that often occurs is the color change to brown as a result of the process of stripping, cutting or exposed to collisions, this process is called the enzymatic browning reaction [7]. Browning is caused by the oxidation of phenolic compounds in the fruit which is catalyzed by the enzyme PPO when the fruit is damaged by cell structure and then produces quinone compounds, these compounds which cause the color to brown[8]. Browning in fruits can reduce product quality and reduce consumer interest [9]. Efforts to inhibit the browning reaction in this fruit have been done using enzyme inhibiting chemical compounds such as ascorbic and citric acid, but until now documentation of chemical compound inhibiting enzyme from organic groups, is still very limited in number [10]. This methods leaves a negative impact on the taste due to the treatment. Therefore, it is necessary to have enzyme inhibiting compounds that do not have negative impact on taste. One alternative is *hypoiodous acid* (HIO). HIO is a weak acid that has been widely used for antibacterial and antifungal properties [11].

HIO is a compound formed form the reaction of two substrates, namely hydrogen peroxide (H_2O_2) and KI which are catalyzed by the peroxidase enzyme[11]. Peroxidase enzyme is an enzyme that can catalyze the transfer of H atoms, O atoms, or electrons from one substrate to another [12]. The enzyme can be obtained

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from the results of isolation and purification in several plants[13], one of which is radish. The focus of this study was to determine the pattern of inhibition of browning reactions in salak using HIO.

MATERIALS AND METHODS

Chemical materials and enzyme. H_2O_2 and KI were purchased from Roche (Germany) and 2 mM of those substrates was applied. The *salak* was obtained from local farm form Sleman, Yogyakarta with 5-month harvest age. 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.

Salak sortation. Preparation of *salak* used as sample referred to[14] with modification. *Salak* were harvested from the orchard located in Sleman, Yogyakarta with relatively same harvest age and transported using container box to prevent possible physical injury. The *salak* fruits were manually picked from the bunch and carefully cleaned with brush to remove the dirt on its skin. Unhealthy and bruised fruits were discarded and fruits weighing 60–80 g were selected. After sorting, fruits were placed in 2 different container boxes according to the treatment, sprayed with HIO and with distilled water. All processes were carried out aseptically.

HIO solution preparation. The method from previous research from [15]. HIO solution was made from H_2O_2 (2 mM), KI (2 mM), and peroxidase 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 was ready to use.

Peroxidase enzyme preparation. Preparation of peroxidase enzyme from Daikon radish was following the method from[16] to obtain the crude extract of peroxidase enzyme from natural sources. Radish were washed and cut into small pieces. The cuts were weighed and blended with phosphate buffer (0.01 M, pH 7) in ratio 1:4. The blended radish was filtered with filter cloth to obtain the juice. The juice then centrifuged for 10 minutes with the speed of 10000 rpm using Scilogex DM0412 centrifuge. The supernatant and sediment were separated by filter cloth and the supernatant was used as peroxidase enzyme that produces peroxidase enzyme of 4,5 U/ml.

Application of HIO on *salak*. This procedure was adopted and modified from previous study by[17]. The container boxes used to store the *salak* fruit were sprayed and cleaned with alcohol before use to avoid contamination. Each fruit was aseptically sprayed with and without HIO solution as much as 1 ml. All of the fruits were then put into the container boxes according to the treatment group and covered with plastic wrap, stored in room temperature for 12 days. These fruits were analyzed every 3 days.

Analysis for b* value. Testing of the b* value referred to the method of [18] with modification. The pointed end of the *salak* was cut to size of 1 cm x 1 cm x 1 cm and measured in 3 different areas of each replicate. Data analysis was carried out using Microsoft Excel then the results were presented in scatter chats with tread line and also explained descriptively.

RESULTS

Change in b* Value

The results of changes in b value of *salak* fruit at room temperature, can be seen in Figure 1. The data was in form of spraying treatment of *salak* with HIO and aquades which stored at room temperature.

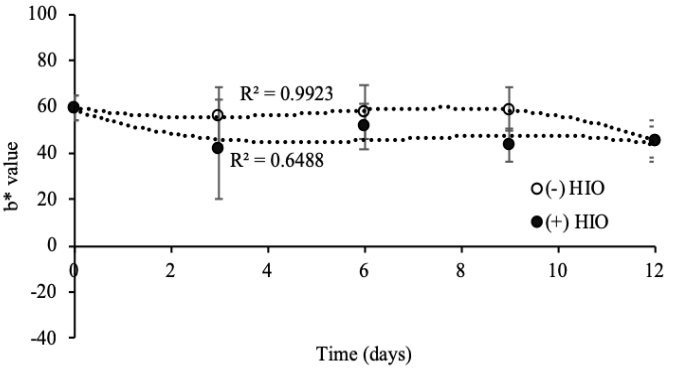

Figure 1. b* Value of *Salak* Stored during Room Temperature

The Michaelis-Menten Curve

Figure 2 means that quadratic rate each of which produces a formula $y= -1.5971x^2 + 20.604x$ and $y= -1.4073x^2 + 17.409x$, respectively for curves without addition and with addition of HIO.

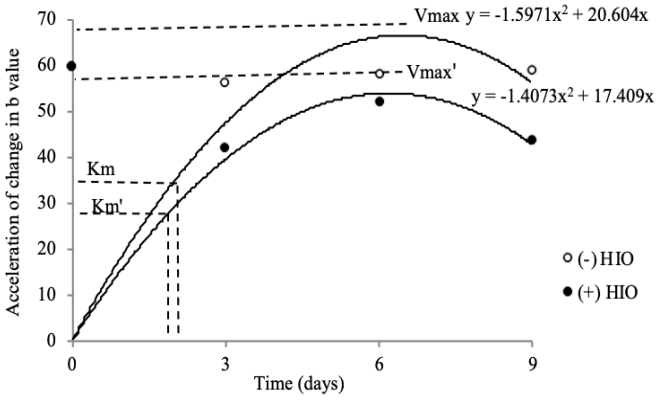

Figure 2. Michaelis- Menten's Curve PPO Enzyme for *Salak*

Lineweaver-Burk Curve for Inhibition Type Determination

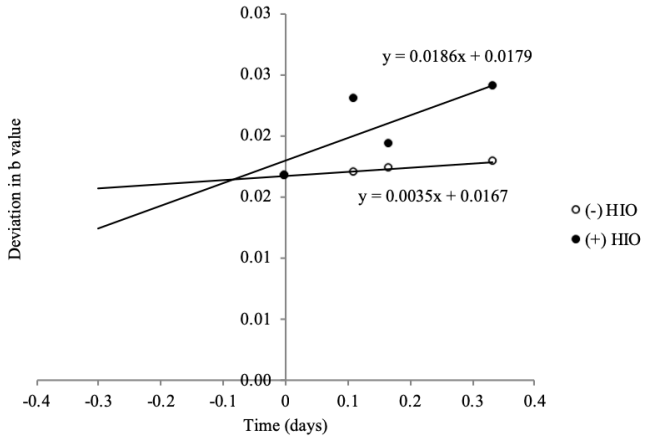

Figure 3. Lineweaver-Burk Plot curve

Enzyme Kinetics

V_{max} and K_m values are generated using graphical analysis as shown in Figure 2, while K_i values are calculated after the known V_{max} and K_m values.

Table 1. PPO Enzyme Kinetic Value Data with HIO Inhibitors

	HIO Inhibitors
K_m	29
$V_{max'}$	58
K_i	13.11
Type of inhibition	Uncompetitive

DISCUSSION

Change in b* Value

Figure 1 showed that b*value of *salak* with HIO treatment has decreased during storage. In general , the graph obtained from non HIO treatment results are lower than HIO treatment. Decrease in b* value is considered fast and can only last for 12 days then *salak* cannot be analyzed further because the discoloration process begins. This is same with previous research which states that the color change on the initial day was no difference, but the color of fruit decreased during storage for less than 14 days, indicating a yellow-brown effect [18]. Brwoning is caused by the oxidation of phenolic compounds in the fruit which is catalyzed by the enzyme PPO when fruit is damaged by the cell structure and then produces quinone compounds, these compounds which cause the color to brown [8]. The b* values of *salak* which treated with HIO has an R^2 value of 0.6488 which indicates that the fruit tends to be far from yellowness color. Although the b* value shows change that tend to be positive, the b* value cannot be used as a direct indicator that affects browning reactions, this is because the b* value has a weak relationship with PPO enzyme activity [15].

The Michaelis-Menten Curve

The Michaelis-Menten Curve is an indicator that can be used to describe changes in enzyme kinetics and to provide inhibitory characteristics for enzyme activity [19]. K_m and V_{max} values are two main parameters in the enzyme kinetics study, each of which represents the Michaelis-Menten constanta which can be used to estimate the number of substrates and acceleration reaction [20]. The acceleration reaction will continue to increase until it reaches the limit point indicating that the activity of this enzyme has reached its maximum limit, this point is called V_{max} [21].

The curve of change in b* value in Figure 2 means that the higher the absorbance value, the more brown the *salak* fruit according to the quadratic rate each of which produces a formula $y= -1.5971x^2 + 20.604x$ and $y= -1.4073x^2 + 17.409x$, respectively for curves without addition and with addition of HIO. The peak that can be reached on each curve is called V_{max}, so V_{max} is the maximumrate that can be achieved in a reaction without the addition of HIO and $V_{max'}$ is the maximum rate that can be obtained in the reaction by adding HIO. Based on the range of V_{max} values obtained, V_{max} of the PPO enzyme with the addition and without addition of HIO occurred on the sixth days that indicating the maximum ability of the PPO enzyme to carry out chemical reaction activities which is characterized by the acceleration of the browning reaction which become slow that ultimately the enzyme cannot function [22]. This Michaelis-Menten curve forms the basis for forming the Lineweaver-Burk curve to determine the value of K_m and V_{max} [23] and also to find out the type of inhibition.

Lineweaver-Burk Curve for Inhibition Type Determination

The browning reaction inhibition mechanism analyzed from the Lineweaver-Burk plot curve (Figure 3) shows that the type of inhibition of HIO enzymes in *salak* is uncompetitive inhibitor. [24] explains that uncompetitive inhibitors have no intersection points on the x or y axis. The mechanism of inhibition differs depending on the inhibitor used. HIO can bind the allosteric side of PPO enzyme that arise when the enzyme binds to the enzyme in the enzyme-substrate complex and can inhibit its activity[25].

Enzyme Kinetics

Table 1 shows the kinetic values of HIO inhibitors with K_m, $V_{max'}$ and K_i values are 29; 58 and 13.11 The Vmax value shows the level of enzyme saturation by the substrate while Km shows the catalyst efficiency of the enzyme which is seen as the concentration of a particular substrate when the catalytic velocity of the enzyme reaches half V_{max} [21]. A low inhibition constant value indicates the strength of the inhibitor inhibits the enzyme activity of each unit [25].

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The constant value obtained is equal to the value of the citric acid constant which is equal to 13 [26].

CONCLUSION

The conclusion obtained from this study is HIO is able to maintain *salak* from enzymatic browning reaction, Inhibition ability increases with increasing concentrations of HIO. The mechanism of HIO inhibition is type of uncompetitive inhibition.

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ABBREVIATIONS

PPO : polyphenol oxidase
HIO : hypoiodous acid
H_2O_2 : hydrogen peroxide
KI : potassium iodide
K_m : michaelis konstant
V_{max} : the maximal rate of reaction with inhibitors
$V_{max'}$: the maximal rate of reaction without inhibitors
K_i : quantitative measure of potential inhibitors

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