Inhibitory Action of Hypoiodous Acid (HIO) against Browning on Apple through the Analysis of Lightness Appearance

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Abstract. Reaction of enzymatic browning occurs due to the oxidation of phenol involving polyphenol oxidase (PPO). Prevention of browning reactions can be done by inhibiting PPO activity, one of the alternative is hypoiodous acid (HIO). The purpose of this study is to know the inhibitory activity of HIO on the enzymatic browning of apple based on the color change. The color change was analyzed by L* value differences. HIO was provided from the reaction between H2O2 and KIO3 using peroxidase as catalyst. Apple was immersed into the solution of HIO for six hour and the lightness was analyzed using digital color meter. Result indicated the decrease of the lightness was found in apple when the immersion in HIO was applied. The inhibitory action of HIO was comparable with NaCl immersion where HIO with concentration of 0.09 had better inhibitory action than HIO 0.068.

1. Introduction

Apple is one of the most popular fruits because it has the potential agent as a source of antioxidants [1, 2]. Apples also contain lots of vitamins and essential minerals for humans such as vitamins A, B1, C, and several types of minerals such as calcium, phosphorus, and iron [3]. Apples also have high phenolic content [4] and have potential anti-diabetes supplement [5]. However, browning reactions in apples are common problem [6] that occur due to enzymatic phenol oxidation [7] involving polyphenol oxidase (PPO) enzymes [1] with polyphenol substrates such as catechins, caffeic acid, pyro catechols/catechol and chlorogenic acid [8]. This reaction changes sensory properties, reduce nutritional quality [9] and reduce consumer acceptance [10, 11]. The brown color caused by the enzymatic browning reaction occurs due to the appearance of quinones which are dark brown color [12, 13].

PPO inactivation methods to inhibit browning reactions can be carried out through thermal processes but may cause tissue softening. The use of modified atmospheric packaging may also be used to prevent browning reactions [14]. Chemical methods using ascorbic acid, cysteine, citric acid,
sodium chloride and benzoic acid are also likely to be carried out but may have a negative impact on taste [15, 16]. The action to prevent browning reactions without providing negative impacts on food quality are still under development. The strength of action in inhibiting browning reactions also needs to be continued in order to determine the type of inhibitor for optimal results.

HIO is a weak acid compound formed from the reaction of H2O2 and KIO which is assisted by peroxidase enzymes as catalysts [17] and able to produce hypoiodite (OI-) or HIO [18]. Peroxidase enzymes are capable to catalyze H2O2 to be H2O and O- [19] and it can be obtained from plants [20]. Besides HIO, enzymatic reactions using peroxidase have also been carried out to produce antibacterial compounds such as HOSCN [21].

Regarding to the safety, HIO has been widely used to inhibit microbial spoilage on tap water [22]. Although it has been well developed as disinfectant in water including drinking water, no provided data were found toward it’s adverse effect to human health. Previous research also mentioned that HIO was not cytotoxic based on the use to well-differentiated epithelial cells in vitro [23].

Based on the literature search, there was no research so far that focuses on the study of the inhibition action of browning on apples using HIO. Therefore, this study aims to determine the ability of HIO to inhibit browning reactions and find out the type of power of inhibition based on lightness changes. This study provides the information on the ability of inhibition of browning reactions using HIO.

2. Materials and methods

2.1. Chemicals and enzyme

This study used “Red Delicious” apple that was obtained from the modern market in the Tembalang area, Semarang, nearby campus. HIO was obtained from the reaction between H2O2 (Merck, Germany) and KI (Merck, Germany) and catalyzed by Horseradish peroxidase (EC 1.11.1.7) (Sigma, Germany, Lot No. K48238016). Measuring cups, beaker cups, stainless steel knife, micro tubes, vortex (Scilogex MX-5) were used as additional tools. Apple digital color meter software and iSight camera were used to determine the lightness under the mini photo studio with 50 lumen lightening.

2.2. Apple Fruit Preparation

The apple preparation procedure was carried out using method of [24] with the following procedure: apples were cleaned from the skin using a stainless steel knife. Latex gloves were used in this process to prevent direct contact with fruit. The peeled apple was then cut to a size of 1.0 x 1.0 x 0.5 cm then was prepared to be immersed in HIO solution.

2.3. HIO Solution and Comparative Solution Preparation

The procedure for making HIO solution was based on the method of other researcher [19]. The process of making HIO solution was carried out by reacting H2O2, KIO3 at concentration of 0.068 mM and 0.09 mM for each then the reaction mixture was added by 20 U/ml peroxidase enzyme. Sterile aquadest were used as control. Solution 0.1 M NaCl was also used as an additional information for the ability of HIO in the inhibition of browning reactions. Prior to use, all solutions were filtered using a syringe filter (with a pore size of 0.22 µm).

2.4. Apple Fruit Immersion

The procedure for apples immersion was carried out using the method of other researcher [24]. Fresh cut apples were soaked in 1 ml of solution containing HIO and NaCl for 10 minutes at room temperature. Fresh cut of apple was also immersed in aquadest as control. The solution and aquadest were removed to analyze the lightness changes every 1 hour until 6 hours using digital color meter software.
2.5. Inhibitory Activity by L Value Testing
Testing of the L* value that was carried out refers to the method of previous researcher [25]. Samples of fresh cut apple was soaked in three various immersion liquids and left in open room for 6 hours. The data were taken at 3 points of area in each samples. The value of L* was then calculated for \( \Delta L \) at every hour to produce a graph then to find out the inhibitory of HIO against browning process in apple.

2.6. Data analysis
Lightness value was obtained from 3 replications and calculated using Microsoft Excel 2010 to find out the L* value average and standard deviation. This study used a quantitative descriptive analysis [26].

3. Results and Discussion

3.1. Color changes
Color changes testing may be used to detect changes in food quality, therefore this research used one of color properties: lightness, to analyze the inhibitory action of HIO against apple browning. Changes in the L* value in the beginning of research reached 78.408±0.936, 82.459 ± 1.430, 85.103 ± 1.171 and 84.332±0.593, respectively for aquadest, HIO 0.068, HIO 0.09, and NaCl immersion (Table 1). This value indicates the range of lightness at 6 point. Then the inhibition was unable to be detected clearly since the variation at initial measurement was appeared.

The inhibition of browning reactions in fruit could be appeared by the presence of inhibitors [15]. The brown color in fruits was appeared due to tyrosinase that converted L-dopa to dopa which formed the brown color of quinone polymers [22, 26]. Therefore the formation of brown color has a close correlation with enzyme inhibitory activity (in this case, PPO), so the brown indicator could also be used as an indicator for enzyme inhibition in fruit browning reactions. Based on the initial value and final value, the HIO succeeded in inhibiting browning reactions of about 7%, while NaCl could inhibit about 6% which means that inhibition of browning reaction by HIO was comparable to that of NaCl at the concentration used in this study.

Table 1. L* values on apples that was immersed in aquadest, HIO, and NaCl.

| Time (Hour) | Immersion Types |
|------------|-----------------|
|            | HIO 0           | HIO 0.068       | HIO 0.09        | NaCl 0.1       |
| 0          | 78.408 ± 0.936  | 82.459 ± 1.430  | 85.103 ± 1.171  | 84.332 ± 0.593 |
| 1          | 76.631 ± 0.926  | 80.781 ± 1.529  | 83.547 ± 1.362  | 82.890 ± 0.448 |
| 2          | 75.104 ± 0.823  | 79.469 ± 1.042  | 82.349 ± 0.857  | 81.788 ± 0.766 |
| 3          | 74.181 ± 0.603  | 78.559 ± 0.603  | 81.438 ± 0.945  | 80.789 ± 0.404 |
| 4          | 73.318 ± 0.332  | 77.697 ± 0.492  | 80.571 ± 0.516  | 80.318 ± 0.688 |
| 5          | 72.739 ± 0.312  | 77.127 ± 0.312  | 79.958 ± 0.430  | 79.723 ± 0.572 |
| 6          | 72.183 ± 0.356  | 76.671 ± 0.434  | 79.767 ± 0.436  | 79.328 ± 0.405 |
| 7          | 71.993 ± 0.365  | 76.548 ± 0.386  | 79.588 ± 0.243  | 79.216 ± 0.427 |

3.2. \( \Delta L^* \) value differences
Based on the results of the study, the highest \( \Delta L^* \) value was 1.777 then followed by 1.678, 1.556 and 1.442 that was achieved by aquadest, HIO 0.068, HIO 0.09, and NaCl, respectively (Fig. 1). The greater of decrease in L* value, the more browning reactions might be occurred [24]. Based on the trendline, since the measurement was done for 6 hour, then the smallest decrease was appeared on NaCl followed by HIO 0.09, HIO 0.068, and aquadest. Therefore, NaCl was proven to have the smallest difference among other treatments indicating strong inhibition on browning reactions. This
may be explained by the strong ability of NaCl to inactivate PPO and degrade the oxidative phenol compounds [28].

**Figure 1.** The value of ∆L* in apple during 6 h storage after immersion in aquadest, HIO, and NaCl

HIO was appeared to inhibit the browning reaction since the trend line showed in the lower position than that of aquadest but the position was still higher than that of NaCl. HIO might occur as inhibitor of enzymatic activity of PPO by binding in the active site of PPO resulting in the inhibition of generation quinone in the apple, thus hindered the browning color appearance of apple during six hour storage.

4. **Conclusion**

Enzymatic browning reactions in apples was succeeded to be determined by analysis of ∆L of apple during storage after immersion in HIO. The value of ∆L concluded that HIO inhibited the browning process in apple. where HIO with concentration of 0.09 mM had better inhibition than HIO 0.068 mM and aquadest. However, the inhibition ability of HIO was lower than NaCl based on the ∆L. Further study regarding residue analysis and organoleptic quality of the apple slices treated with HIO solution also needed to be done to provide more information about HIO safety and effect towards foods.

5. **References**

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