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## Determination Hypoiodous Acid (HIO) By Peroxidase System Using Peroxidase Enzyme

A N Al-Baarri<sup>1,3</sup>, A M Legowo<sup>1</sup>, Widayat<sup>2,3</sup>, S B M Abduh<sup>1</sup>, M Hadipernata<sup>4</sup>, Wisnubroto<sup>4</sup>, D K Ardianti<sup>1\*</sup>, M N Susanto<sup>1</sup>, M Yusuf<sup>1</sup>, E K Demasta<sup>1</sup>

<sup>1</sup>Food Technology Department, Faculty of Animal and Agricultural Sciences, Diponegoro University, Semarang, Indonesia.

<sup>2</sup>Chemical Engineering Department, Faculty of Engineering, Diponegoro University, Semarang, Indonesia

<sup>3</sup>Laboratory of Food Technology, UPT Integrated Laboratory, Diponegoro University, Semarang, Indonesia

<sup>4</sup>Center of Agricultural Post-Harvest Research and Development, Bogor, Indonesia

E-mail: albari@undip.ac.id

**Abstract.** It has been understood that peroxidase enzyme including peroxidase serves as catalyzer to enzymatic reaction among hydrogen peroxide and halides, therefore this research was done for generating hypoiodous acid (HIO) from peroxidase system using peroxidase enzyme. Hydrogen peroxide, potassium iodide, and peroxidase enzyme were used to produce HIO. Determination the amount of formed HIO was done using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS as substrate through the colorimetric measurement of hydrogen peroxide residue during reaction process using at 412 nm. The result indicated that residual hydrogen peroxide showed the minimum concentration after 60 minutes reaction time. Because the reaction started at the beginning time of mixing, hydrogen peroxide was unable to be eliminated totally to produce HIO. The reaction of peroxidase system was able to determine the beginning of mixing process but the reaction process could not eliminate the initial concentration of hydrogen peroxide indicating the maximum amount of production of HIO could be determined. In conclusion, the less of H<sub>2</sub>O<sub>2</sub>, higher HIO obtained and peroxidase enzymes can accelerate the formation of HIO.

**Keywords:** Peroxidase system, hypoiodous acid, residual hydrogen peroxide, iodate, ABTS.

### 1. Introduction

Enzymes are known as good catalyzer. One of important enzyme in food is peroxidase. Peroxidases are widely distributed in nature and can be extracted from most plant cells, some animal tissues and fungus [1]. Plant peroxidase is an enzyme that catalyzes the one electron oxidation of various organic and inorganic substrates in the presence of hydrogen peroxide [2]. Plant peroxidases have been isolated and purified from several plant sources, such as *Leucaena leucocephala*, *Viscum angulatum*, *Vigna mungo*, *Solanum melongena*, *Beta vulgaris*, *Roystonea regia*, *Tamarix*, *Jatropha curcas* [3]. Peroxidase enzyme is one of the enzymes that can catalyse reaction between substrate and oxygen molecule. Peroxidases are much less effective as catalysts of oxygen-transfer reactions than cytochromes P450 [4]. Iodine is a compound of anion halide or (X<sup>-</sup>), IO<sup>3-</sup>, so application peroxidase can also catalyse the combination of hydrogen peroxide (H<sub>2</sub>O<sub>2</sub>) and potassium iodate (KIO<sub>3</sub>) to produce Hypoiodite (OI<sup>-</sup>) or Hypoiodous Acid (HIO).



Peroxidase enzyme activity test was generally performed using UV-VIS spectrophotometer with 412 nm wavelength and using 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) or ABTS as substrate [5]. ABTS was used as an indicator because it was easily transformed into a radical form. ABTS oxidized with  $H_2O_2$ , then would form a radical compound of metastable cations and blue or green chromophore [6]. This research was done to analyse concentration of hypoiodous from the reaction mixture of  $H_2O_2$  and  $KIO_3$ . Since hypoiodous has antibacterial properties, the minimum concentration of substrates  $H_2O_2$  and  $KIO_3$  was very important in order to generate maximum HIO through peroxidase system.

## 2. Materials and Methods

### 2.1. Chemicals and enzyme

$H_2O_2$  (0.15 mM), KI (0.15 mM), 2,2'-azino-bis [3-ethylbenzothiazoline-6-sulphonic acid] (ABTS) and horseradish peroxidase were obtained from Roche (Germany). Aquadest was obtained from UPT Integrated Laboratory, Diponegoro University, Semarang, Indonesia.

### 2.2. Preparation of HIO

There were two methods for determination of HIO. A 450  $\mu$ l of 0.15 mM  $H_2O_2$  was mixed with 450  $\mu$ l of 0.15 mM KI and 100  $\mu$ l of enzyme peroxidase. The other one was 450  $\mu$ l of 0.15 mM  $H_2O_2$  was mixed with 450  $\mu$ l of 0.15 mM KI and 100  $\mu$ l of aquadest. Solution was reacted, then measured using ABTS method every 10 minutes for an hour. This method was adopted from previous researcher [5]. The mixture of  $H_2O_2$  and KI was determined as HIO solution.

### 2.3. Residue $H_2O_2$ with ABTS method

A 450  $\mu$ l of HIO solution was added to a reaction mixture containing 450  $\mu$ l of 0.55 mM ABTS in Phosphate Buffer (0.1 M, pH 7) and 100  $\mu$ l of horseradish peroxidase (4.5 U). ABTS as substrate through the measurement of hydrogen peroxide residue during reaction process using spectrophotometer at wavelength of 412 nm. This method was derived from previous researcher [7].

## 3. Result and Discussion

### 3.1. Residue of $H_2O_2$

Measurement of  $H_2O_2$  residues is enabled to determine the acceleration of HIO formation using peroxide enzyme catalyst or not using a catalyst as shown at Figure 1 and 2. The residue of  $H_2O_2$  was measured by the absorbance value of the HIO solution using ABTS as substrate. The addition was intended for produces a green colour of the reaction between the rest of  $H_2O_2$  and ABTS. The thicker the green colour the higher of absorbance. The decrease of absorbance value could be interpreted as the acceleration of HIO acid formation. Based on Fig. 1 and 2, it can be seen that the reaction between  $H_2O_2$  and KI without enzyme run slower than those of enzyme addition. The velocity reaction of both compounds could be seen at the interval of spectrophotometric absorbance. This is because enzymes accelerate the reaction process of the formation of new compounds and the new compound from that reaction was HIO. This is in accordance with the opinion from other researcher [8] which states that peroxidase enzyme as catalysts of the oxidation of iodide into HIO. The more enzymes were given, the faster the reaction changes may occur. The speed of reaction may also increase with increasing concentration enzyme.

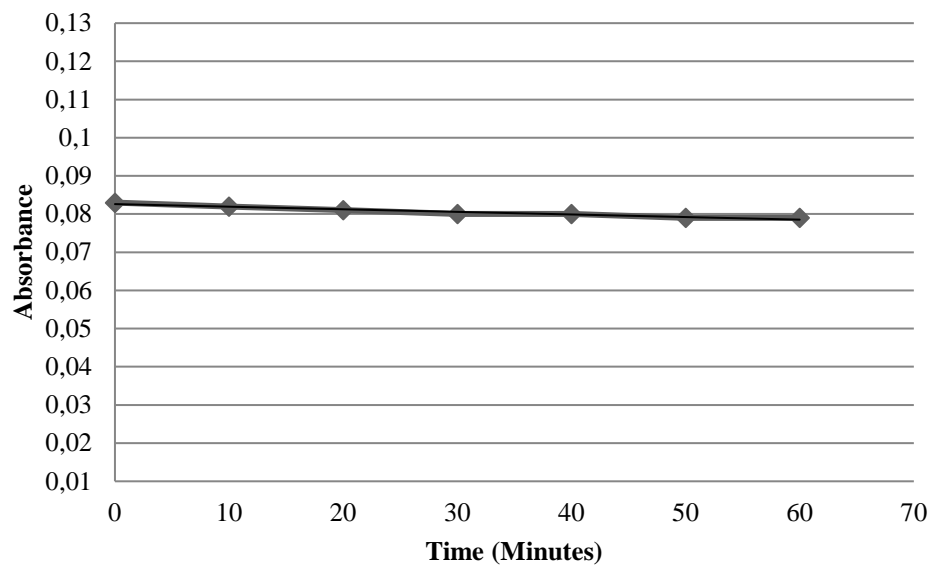


Figure 1. Residue  $H_2O_2$  without Enzyme Peroxidase

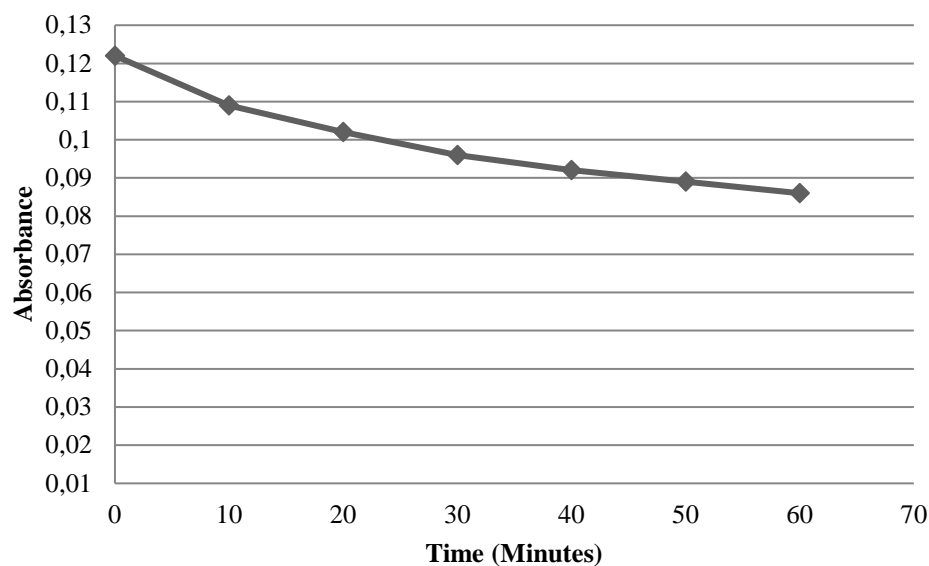


Figure 2. Residue  $H_2O_2$  with Enzyme Peroxidase

The concentration of enzymes was expressed in units of units (U) and this research used 4.5 U. This amount of concentration was determined as eligible concentration to analyse the rate of reaction. As time of reaction, the enzyme might stop working resulting the decrease in reaction speed. The initial reaction speed generated large value then followed by smaller concentration of substrate's reduction. This can be seen at Table 1 where at the absorbance of  $H_2O_2$  residue with enzyme was 0.092 to 0.089 in ten minutes starting from 40 to 50, then the reaction started to run slowly. Based on Table 1, the higher decrease in residual value of  $H_2O_2$  indicated the more HIO was formed, meanwhile the absorbance of residue  $H_2O_2$  without enzyme showed that the high residual value of  $H_2O_2$  and it indicated that the HIO formed was still less in concentration. This is in accordance with the opinion from other researcher [5] which states that as the decline in the value of hydrogen peroxide concentration and the halide ions used in the peroxidase system, the higher concentration in final product of peroxidase systems.

### 3.2. Accelerated Reaction of Residual $H_2O_2$

Table 1. Accelerated Reaction Results of Residue  $H_2O_2$  With and Without Enzyme Peroxidase

Time (Minutes)	Enzyme (Absorbance)	Acceleration of reaction	Non Enzyme (Absorbance)	Acceleration of reaction
0	0.122	0.013	0.083	0.001
10	0.109	0.007	0.082	0.001
20	0.102	0.006	0.081	0.001
30	0.096	0.004	0.08	0
40	0.092	0.003	0.08	0.001
50	0.089	0.003	0.079	0
60	0.086		0.079	

One of the factor that might affected the formation of HIO compound was the composition of the substrate. This was due to the peroxidase enzyme's ability to catalyse the oxidation reaction of KI with  $H_2O_2$ . According to other researcher [9], lactoperoxidase would be actively optimized when a state of sufficient ionic halide or pseudohalide. Meanwhile, the presence of an abundant substrate was also not providing the high concentration of HIO. According to previous researcher [10], the redox reactions existed in the peroxidase system could only occur if the reduction capability of the enzyme was weaker than the reduction capability of the substrate used. In addition to these factors, several things might also affected redox reactions in peroxidase systems such as anion size, anion access and anion ability to bind [11]. Besides these factors, the acidity level could also affect the reaction between  $H_2O_2$  and KIO. According to other researcher [12], the acidity might hinder the action of hydrogen peroxide to oxidise iodine.

### 4. Conclusion

The peroxidase enzyme might accelerate the reaction process of peroxidase system for generating HIO and resulting minimum concentration of residual substrate. The maximum amount of production of HIO could be determined through the minimum rate of reaction that was reached at minute 40<sup>th</sup> of reaction time.

### References

- [1] Balasubramanian, M and R Boopathy 2013 *Molecular catalysis B: Enzymatic* **90**: 33-42
- [2] Bozzo GG, K G Raghothama, and WC Plaxton 2004 *Biochem. J.* **377**: 1369-1379
- [3] Pandey VP and UN Dwivedi 2011 *Journal of Molecular Catalysis B: Enzymatic.* **68** (2): 168-173
- [4] Veitch NC 2004 *Phytochemistry* **65**: 249-259
- [5] Touch V, S Hayakawa, S Yamada and S. Kaneko 2004 *J. of Food Microbiology* **93**: 175-183.
- [6] Glucin I and A Dastan 2007 *Enzyme Inhibition and Medicinal Chemistry* **22**(6): 685-695
- [7] Al-Baarri AN, M Hayashi, M Ogawa and S Hayakawa 2011 *Journal of Food Protection* **74**: 134-139
- [8] Kupper FC, N Schweigert, E Ar Gall, JM Legendre, H Vilter and B Kloareg 1998 *Planta.* **207**: 163-171. Doi
- [9] Seifu E, E M Buys and E F Donkin 2005 *Trends in Food Science and Technology* **16** (4): 137-154
- [10] Bafort F, O Parisi, JP Perraudin and MH Jijakli 2014 *Enzyme Research* 1-13.
- [11] Davies MJ, CL Hawkins, DI Pattison and MD Rees 2008 *Antioxidants & Redox Signaling* **10** (7): 1199-1234
- [12] Schmitz G 2001 *Phys. Chem.* **3**: 4741-4746