

## **Effect of temperature on guaiacol Peroxidase of *Pyrus communis***

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### **ABSTRACT**

Peroxidase (EC 1.11.1.7; donor: hydrogen-peroxide oxidoreductase, POD) is one of the key enzymes controlling plant growth, differentiation and development. The enzyme participates in construction, rigidification and eventual lignification of cell walls, biosynthesis of ethylene from 1-aminocyclopropane-1-carboxylic acid and H<sub>2</sub>O<sub>2</sub>, regulation of auxin level through auxin catabolism, protection of tissue from damage and infection by pathogenic microorganisms, the oxidation of indoleacetic acid. For peroxidase activity in wild pears extract one pH optimum was observed at 6.5 that probably belong to atleast one isoenzyme. Activity of peroxidase in presence of guaiacol and H<sub>2</sub>O<sub>2</sub> was optimum after incubation at 40 °C. Maximum activity of peroxidase is 300 % .Activity increased to 240 %, 300 %, 70 % and 10 % after 60 minute incubation at 30, 40, 45 and 60 °C for peroxidase. Incubation at high temperature (70 °C) was accompanied with decrease of activity to 10 % peroxidase activity.

**Keyword:** guaiacol; peroxidase; *pyrus communis*; temperature

### **1. INTRODUCTION**

Peroxidase (EC 1.11.1.7; donor: hydrogen-peroxide oxidoreductase, POD) is one of the key enzymes controlling plant growth, differentiation and development. The enzyme participates in construction, rigidification and eventual lignification of cell walls, biosynthesis of ethylene from 1-aminocyclopropane-1-carboxylic acid and H<sub>2</sub>O<sub>2</sub>, regulation of auxin level through auxin catabolism, protection of tissue from damage and infection by pathogenic microorganisms, the oxidation of indoleacetic acid (Dunford 1991; Wakamatsu and Takahama, 1993). *In vitro*, this enzyme is widely employed in microanalysis (Krell, 1991). More than 80 % of immunoenzymatic kits contain peroxidase as labeling enzyme. Recently, peroxidases have been used for biotransformation of organic molecules (Adam *et al.*, 1999). It has been well established that peroxidase as one of the most stable enzymes can contribute to deteriorative changes in quality of the processed products (Stanley *et al.*, 1995). Enzymatic browning is one of the most important colour reactions that affect fruits and vegetables.

It is catalyzed by polyphenoloxidases and peroxidases (Jiang *et al.*, 2004). It was estimated that over 50 percent losses in fruits and vegetables occur as result of enzymatic browning (Whitaker and Lee, 1995). Marshall *et al.* (2000) reported that the rate of enzymatic browning in fruits and vegetables is governed by the active polyphenoloxidase and peroxidase content of the tissues, the phenolic content of the tissues, pH, temperature and oxygen availability with the tissue. According to Khan and Robinson (1993) the peroxidase is directly responsible for enzymatic browning in mangoes. The objective of this work was to study the effect of pH and heat on peroxidase activity and to extend the knowledge of vegetables peroxidase in particular.

## 2. MATERIALS AND METHODS

For extract preparation, wild pears were used throughout these studies. Extracts were prepared from pears weighting each between 50 and 80 g by homogenization in phosphate buffer 0.1 M, pH 7 in presence of PMSF 2 %. After centrifugation at 3,000 g for 10 min, then at 35,000 g for 30 min, a clear, transparent supernatant termed “crude extract” was obtained and used for our studies. Protein concentration was determined by the Lowry method (Lowry *et al.*, 1951).

### 2. 1. Effect of pH

pH profile of peroxidase activities was determined spectrophotometrically at 25 °C by measuring the appearance of reaction products in the medium. The activity of the enzyme in wild pears were determined in the pH range 3-10 by using a citrate-phosphate-borate buffer 0.1 M. The optimum pH for PPO activity of extracts was obtained in presence of guaiacol (20 mM) and H<sub>2</sub>O<sub>2</sub> (10 mM) as substrates.

Enzymatic activity was determined by measuring the increase in absorbance at 420 nm with a spectrophotometer (6305 JENWAY). Assays were carried out by addition of 200 µl of extracts to the sample cuvette, and changes in absorbance were recorded. The reference cuvette contained just 3 ml of substrate solution. Enzyme activity was calculated from the linear portion of the curve. One unit of PPO activity was defined as the amount of enzyme that produces 1 micromole of product per minute. Assays were carried out at room temperature and results are the averages of at least three assays and the mean and standard deviations were plotted.

### 2. 2. Effect of Temperature on peroxidase activity in wild pear

pH profile of *Pyrus communis* Peroxidase led to a peak at 6.5 that probably belong to atleast one isoenzyme. For determining the optimum temperature values of the enzyme, peroxidase activity was measured, at constant temperatures (27, 30, 40, 45, 50, 60 and 70 °C separately) using guaiacol (200 mM) and H<sub>2</sub>O<sub>2</sub> (10 mM). The effect of temperature on the activity of peroxidase was tested by heating the crude extract to the appropriate temperatures in different times.

The desired temperatures were provided by using a Memmert model ST/70 temperature controller attached. After different times (0-60 minute) at a same temperature, enzyme cooled in ice and was added and the reaction was followed spectrophotometrically at given time intervals as described above.

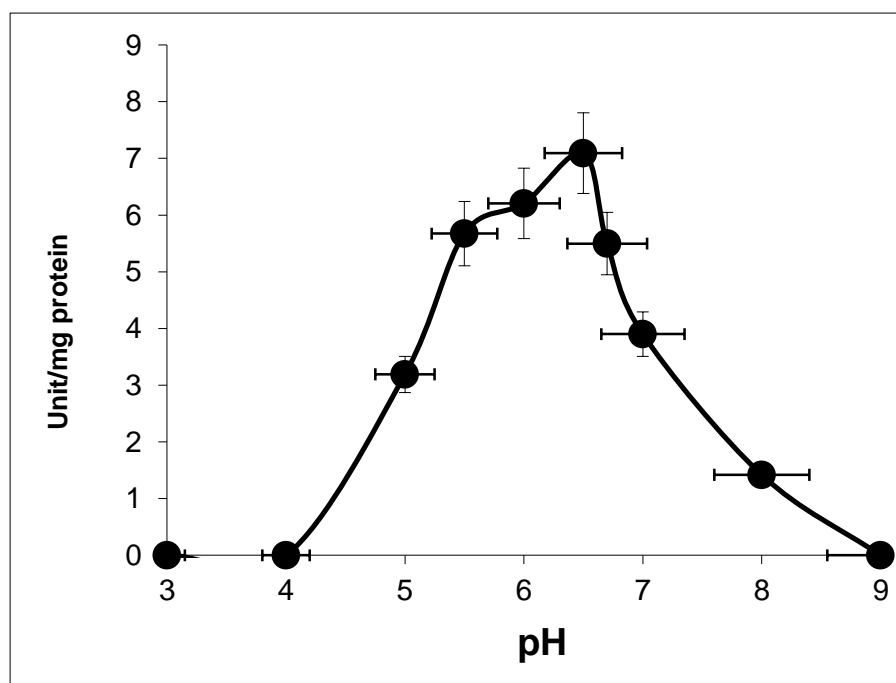
### 2. 3. Heat Inactivation of peroxidase

The thermal denaturation of the peroxidase was studied at different temperature (30-90 °C) and constant concentration of guaiacol 20 mM and H<sub>2</sub>O<sub>2</sub> 10 mM. 200 µl of crude extracts solution in a test tube was incubated at the required temperature for fixed time intervals. At the end of the required time interval, the test tube was cooled in an ice bath. The activity of the peroxidase was then determined.

## 3. RESULTS AND DISCUSSION

### 3. 1. pH profile of peroxidase activity in *Pyrus communis*

pH is a determining factor in the expression of enzymatic activity; it alters the ionization states of amino acid side chains or substrate. For peroxidase activity, one pH optimum were observed at 6.5. No activity was detectable at pH 3 and 9, regardless of the condition. Figure 1 shows the pH activity profile obtained for peroxidase.

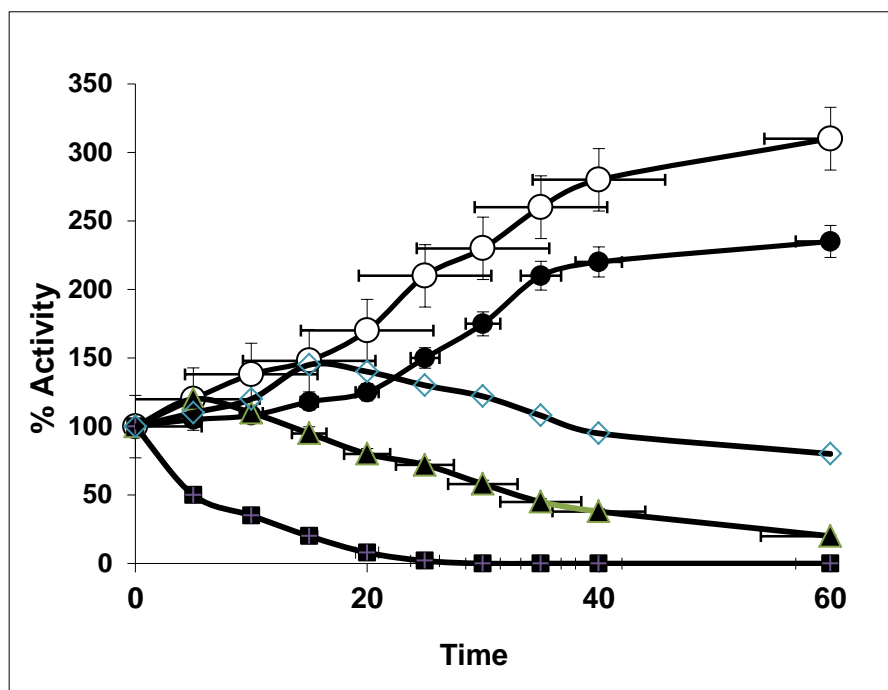


**Figure 1.** The pH dependence of peroxidase activity of *Pyrus communis*. Activity was determined in 0.1 M sodium-acetate buffer and phosphate buffer system in the presence of 20 mM guaiacol and 10 mM H<sub>2</sub>O<sub>2</sub>.

### 3. 2. Effect of temperature on peroxidase activity

Time courses at 30 °C showed an increase of peroxidase activity so, after 60 minute incubation, activity reached to 240 % (Fig. 2). Time course at 40 °C has the similar condition, so increase in time of incubation accompanied with more increase in activation. After 60 minute incubation of peroxidase in 40 °C, activity reached to 300 %. Time course at 45 °C has the different results, so increase in time of incubation accompanied with more increase in activation up to near 20 minute. Increase in time of incubation more than 20 minute up to 60

minute accompanied with decrease in activation, so after 60 minute incubation of peroxidase in 45 °C, activity decreased to 70 %. Time course at 50 and 60 °C showed different behavior for peroxidase, so increase in time of incubation accompanied with more decrease in activation. After 60 minute incubation of peroxidase in 50 and 60 °C, activity reached to 10 and 0 %.



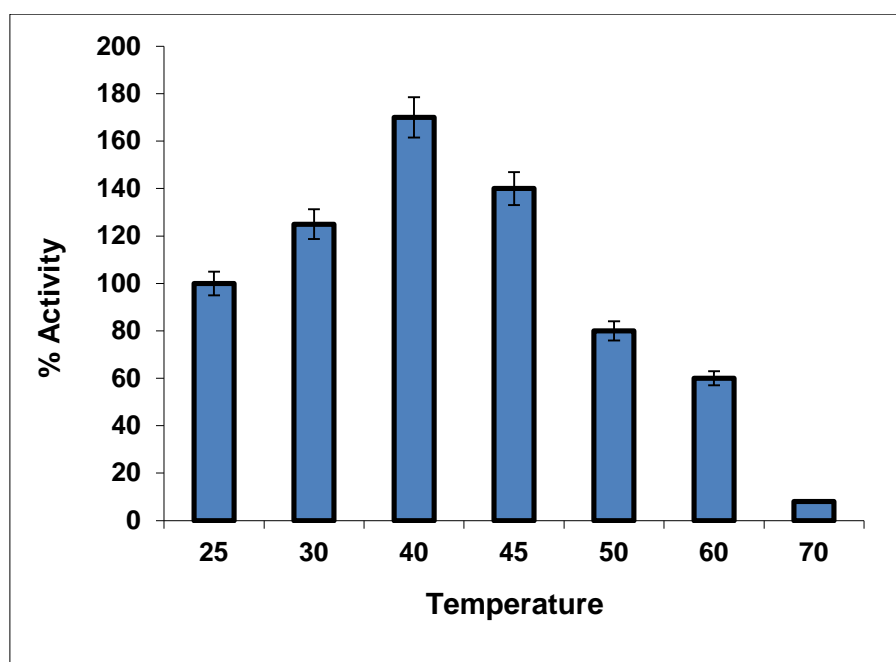
**Figure 2.** Effect of time of incubation on activity of peroxidase in *Pyrus communis* in presence of guaiacol 20 mM and H<sub>2</sub>O<sub>2</sub> 10 mM [ 30 °C (●) and 40 °C (○), 45 °C(◇), 50°C(▲ ) and 60 °C(■)].

We can conclude that increase in temperature from 30 to 40 °C change probably structure of peroxidase to ordered shape, so that activity of enzyme in 30 and 40 °C increased and in more temperature (60 and 70 °C) enzyme slowly denature and activity decreased. Activity of peroxidase after 60 minute incubation at 40 °C is 1.5, 5 and 33 times more than 30, 45 and 60 °C.

### 3. 3. Optimum Temperature and Thermal Inactivation

PPO in presence of guaiacol and H<sub>2</sub>O<sub>2</sub> showed fluctuations in activity with increasing temperature even as high as 70 °C. The plot for guaiacol demonstrated that the enzyme was very thermostable between 25 and 45 °C. Our results showed optimum temperature is 40 °C for both peroxidase activity. Like most chemical reactions, with increase of temperature from 27 °C, gradually, activity of peroxidase increased so; we reached to maximum of activity at 40 °C (300 %) (Fig. 3). With more increase in temperature from 40 °C to 70 °C, activity of peroxidase decreased gradually and reached to 10 % at 70 °C. The drop in percentage residual activity at high temperatures can actually be due to the unfolding of the tertiary structure of the enzyme to form the secondary structure. Activity of peroxidase even in 70 °C was observed and is 10 % related to control (25 °C). These results showed that, peroxidase show activity at high temperatures. A ten degree Centigrade rise from 40 to 50 °C in temperature

after 5 minute incubation will increase the activity of peroxidase from 170 to 70 %. Variations in reaction temperature as small as 10 degrees from 30 to 40 °C introduce increase of 50 % in the activity and from 60 to 70 °C introduce decrease of 50 % in the activity.



**Figure 3.** Effect of temperature on activity of peroxidase in extract of wild pear (*pyrus communis*) in presence of guaiacol 20 mM and H<sub>2</sub>O<sub>2</sub> 10 mM.

#### 4. CONCLUSION

For peroxidase activity, this is complicated by the fact that activity of enzymes is adversely affected by high temperatures. As shown in Figure 2 and 3, the reaction rate increases with temperature to a maximum level, then abruptly declines with further increase of temperature. Because this enzyme rapidly become denatured at temperatures above 40 °C in contrast to most of enzymes that denatured at temperatures above 40 °C. peroxidase is very resistant at high temperature such 45 °C, so that peroxidase remain 40 % of its activity at 50 °C. These results showed that potential of activation for peroxidase is very high. It has been noted that heat stability of the enzyme may be related to ripeness of the fruit and molecular forms of the enzyme, and in some cases it is also dependent on pH (Zhou and Feng, 1991). Rapid decrease in activity in high temperature after optimum temperature might be due to involvement of disulfide bond in the active site or in three dimensional conformation of the enzyme. There are several other reports that describe high temperature stability of peroxidase from other sources to the same temperature range. The drop in percentage residual activity at high temperatures can actually be due to the unfolding of the tertiary structure of the enzyme to form the secondary structure (Lowrenco et al., 1990).

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**Shahryar Saeidian** is Associate Professor of Payame Noor University. He teaches biochemistry, molecular biology, enzymology and advanced biochemistry of proteins and nucleic acids. Saeidian's research focuses on the investigation of activity of enzymes, ligand binding of proteins and purification of enzyme. One strand of research examines the activity of polyphenol oxidase and peroxidase in plants. Saeidian received his Ph.D. degree in 2006 from Institute of Biochemistry and Biophysic (IBB) of Tehran University and has a BA and MA in biology and biochemistry at Shiraz and Tehran University (Iran). Shahryar is from Iran and was born in Kurdistan (Baneh). Dr. Saeidian has published over 15 articles in the areas of enzymology and ligand binding. He is a member of the Biochemistry Society and Asia-Pacific Chemical, Biological & Environmental Engineering Society (APCBEEES). He has been as a referee and a member of technical committee of many of national and international congress.

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