

Peroxidase properties of fresh-cut potato browning

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Abstract. The browning of fruits and vegetables during processing is mainly induced by relevant enzymes and phenolic substances. Potato is a typical material easy to brown, however there is no system research about the peroxidase (POD) properties in potato. In this study, it was shown that the optimal pH of POD was 6.0. The optimal temperature of POD was 55°C. POD activity was strongly suppressed by 0.02% L-cys and 0.08% ascorbic acid. The results will provide the theoretical basis and practical guidance for the browning inhibition of fresh-cut potato.

1. Introduction

POD is widely found in plant tissues and plays an important part in plant resistance to stress, growth and development, genetic breeding and lignin synthesis [1]. POD is a key enzyme that causes browning of fruits and vegetables, which catalyzes the oxidation of phenols by peroxide and eventually leads to browning of fruits and vegetables [2]. POD activity in the pericarp increased consistently with skin browning index during storage of litchi fruit [3]. Zhan et al. [4] suggested that browning of fresh-cut lettuce was related to POD activity and quinone accumulation.

The above research shows that POD is a very important enzymes related to enzymatic browning of fruits and vegetables. Therefore, the composition and content of potato polyphenols were identified in the study. The reaction kinetics of potato POD was systematically studied by analyzing the optimum pH and temperature, concentration of substrates, and effects of inhibitor. The results of the study will provided the theoretical basis and practical guidance for the inhibition browning in fresh-cut and storage of fruits and vegetables especially potato.

2. Materials and Methods

2.1. Plant Material and Experimental Design

Potatoes were cleaned and cut into 3 mm slices, then were put into 0.03 mm PE plastic bags. The total phenols and free phenols contents were determined. It was systematically studied that the optimal pH and temperature, and temperature tolerances of POD, the effects of different substrate concentration and inhibitors on POD activity.

2.2. Effects of pH on POD Activity

The 0.2 mol/L acetic acid-sodium acetate buffer solutions were prepared with different pH as 2.0, 2.5, 3.0, 3.5, 4.0, 4.5, 5.0, 5.5, 6.0, 6.5, 7.0, 7.5, 8.0, 8.5 and 9.0 in order to measure the POD activity. The potato POD activities under different pH conditions were determined as follows.

The reaction solution included 2 mL 0.2 mol/L pH 5.5 acetic acid-sodium acetate buffer solution, 1 mL 0.05 mol/L guaiacol, 0.1 mL 0.75% H₂O₂ and 0.05 mL enzyme solution. The control was distilled water instead of the substrate in the reaction liquid. The reaction system was placed in the range of



330-500 nm for full wavelength scanning after reaction for 10 min. The absorption value was measured at the maximum absorption wavelength of the product in above reaction system, and recorded every 15s. The one POD (U) was the increased one of absorbance of the enzymatic reaction system per gram per minute. It can be expressed as $1\text{ U} = \Delta\text{OD}_{\lambda\text{max}} / (\text{min} \cdot \text{g m}_f)$.

2.3. Effects of Temperature on POD Activity

The reaction systems of potato POD were placed at 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80°C, respectively, balanced 5min, added POD enzyme extract, then immediately measured the POD activity. The POD activity was respectively assayed according to the relevant method.

2.4. Effects of Substrate Concentration on POD Activity

The substrate were guaiacol, catechol, gallic acid, pyrogallol, chlorogenic acid, caffeic acid and ferulic acid solution, formulated into 0, 4, 8, 12, 16, 20, 30, 40, 60, 80 and 100 mmol/L. POD activity were determined at pH 5.5, 25°C and in the maximum absorption wavelength. The Michaelis constant (K_m) of enzyme in different phenolic substrates and the maximum reaction rate (V_{max}) of enzymatic reaction were calculated by using Lineweaver-Burk double reciprocal method.

2.5. Effects of Inhibitors on POD Activity

L-cysteine (L-cys), citric acid (CA), ethylenediaminetetraacetic acid (EDTA), ascorbic acid (AA), sodium chloride (NaCl) and sodium hydrogen sulfite (NaHSO_3) were prepared with pH 5.5 acetic acid-sodium acetate buffer solutions. The concentrations of the above solutions were 0, 0.02, 0.04, 0.06, 0.08, 0.10 %. The above solutions were used as the reaction media to compare the inhibitory effects of different inhibitors on POD activity. The results were expressed relative enzyme activity. The formulas were as the following.

$$\text{Relative enzyme activity (\%)} = \text{Residual POD activity} / \text{POD activity at normal temperature} \times 100.$$

2.6. Data Handling

There were three replicates per treatment for measurements. Data were subjected to the analysis of variance by using Origin 8.0. The overall least significant difference ($p = 0.05$) was calculated and used to detect significant differences among measurements.

3. Results and Discussion

3.1. Effects of pH on POD Activity in Potato

The effect of pH on POD activity was obvious (Figure 1). POD activity was reached peak at pH 6.0. So the optimum pH of POD in potato was 6.0. When the $\text{pH} > 6.0$ or $\text{pH} < 6.0$, POD activity had dropped significantly ($P < 0.05$). The POD activity was completely disappeared at pH 2.0 or 9.0. For the above efforts, it was shown that strong acid and alkali were bad for the POD performed reaction. Potato had been sensitive to the pH, mainly due to the lowest and highest pH accelerated that the heme groups of POD active site dissociated, resulted in the lost of POD activity [5].

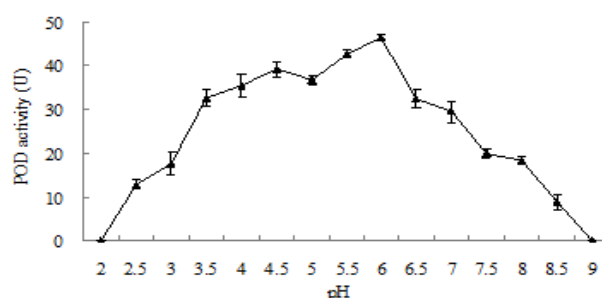


Figure 1. Effects of pH on POD activity in potato. The assay was repeated three times. Bars show standard errors of the means ($n = 3$).

3.2. Effects of Temperature on POD Activity in Potato

As shown in Figure 2, the POD activity appeared the highest peak at 55°C, and appeared another peak at 25°C. It might be that POD in potato was possessed isoenzyme. The optimum temperature for POD was 55°C which was similar to POD of water caltrop pericarp (60°C) [6], while it was different from POD in iranian medlar (35°C) [7], and loquat fruit (35°C) [8]. However, POD activity had been maintained at a lower level from 5 to 15°C. It was shown that low temperature could reduce POD activity and cut down enzymatic reaction. Therefore, potato was suitable to store and process at low temperature. The POD activity increased fastly, and reached a significant level at 15-25°C ($P < 0.05$). The activity of POD was maintained higher level at 50-60°C. But, POD activity had dropped significantly at 60-80°C ($P < 0.05$). It was followed that POD activity was destroyed and passivated at high temperature.

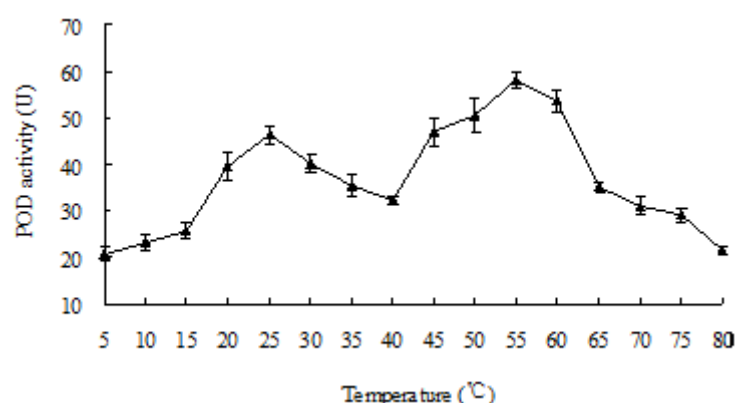


Figure 2. Effects of temperature on POD activity in potato

3.3. Effects of Substrate Concentration on POD Activity in Potato

The activity of POD of potato was impacted greatly by different concentration of phenolic substances (Figure 3). Using gallic acid as substrate, the POD activity was the highest, and when substrate concentration at 8-100mmol/L, the activity of POD was higher than other substrates observably ($p < 0.05$). When substrate concentrations were 0-20mmol/L, the activity of POD rose straightly with the concentration increasing. When concentrations were 20-80mmol/L, POD activity changed very little. When concentration exceeded 80mmol/L, POD activity had downtrend. When guaiacol concentration was 60 and 80mmol/L, POD activity was higher than other substrates obviously, except gallic acid ($p < 0.05$). When concentration was under 60mmol/L, POD activity increased straightly, while POD activity decreased when concentration exceeded 60mmol/L. Using catechol and chlorogenic acid as substrates respectively, the difference of POD activity was clearly ($p < 0.05$). With concentration increasing, the change of POD activity was climbed up and then remained unchanged. The activity of POD reached the highest when 80mmol/L catechol and 60mmol/L chlorogenic acid. Using caffeic acid and pyrogalllic acid as substrates, when concentration exceeded 40mmol/L, POD activity had not obvious difference. Ferulic acid and pyrogalllic acid as substrates, the activity of POD also had not obvious difference. It was shown that the activity of POD was influenced by phenolic varieties and concentrations. The relationship between POD and substrates confirmed to Michaelis-Menten kinetics except gallic acid and guaiacol.

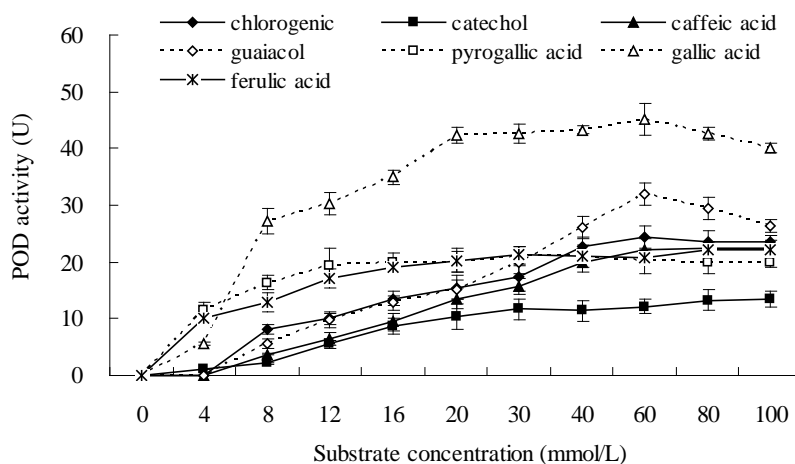


Figure 3. Effects of substrate concentration on POD activity in potato

As shown in Figure 3, POD's binding ability with each substrate in proper order was gallic acid>caffeic acid>guaiacol>chlorogenic acid>catechol>pyrogallol>ferulic acid. The optimum substrate of POD in potato was gallic acid, which was different from POD in loquat fruit [8].

3.4. Effects of Inhibitors on POD Activity in Potato

As shown in Figure 4, different inhibitors had varying degrees of influence on the activity of POD in potato. The activity of POD was strongly suppressed by L-cys, and 0.02 % L-cys could inhibit the 94.3 % activity of POD. The activity of POD was suppressed well by AA. When at low concentration, inhibitory effect of AA was inferior to L-cys. When concentration reached to 0.08 %, POD activity was inhibited completely by AA. Under the experimental concentration, the activity of POD was suppressed unclearly by CA, NaCl, NaHSO₃ and EDTA, and when concentration reached 0.1 %, the residual activity of POD was still more than 89 %. Different inhibitors have different mechanisms of inhibition browning, and different inhibitors have their optimal concentration for inhibiting POD activity [9].

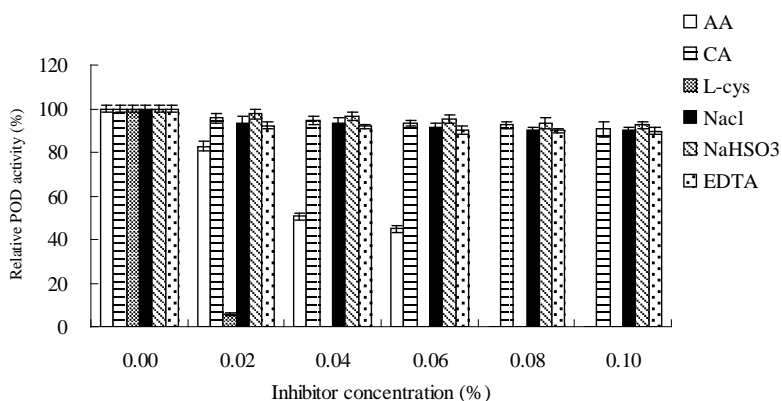


Figure 4. Effects of inhibitors on POD activity in potato

L-cys can be used as a quinone chelating agent to affect quinone, the intermediate product of enzymatic browning, and to form stable colorless compound, so that the product maintain a good appearance [10]. AA can revert oxidized quinones and their derivatives to phenolic substances, to prevent the further polymerization of ketones to form melanoids, but the amount is too large, easy to cause non-enzymatic brown and change the appearance of product [11]. AA as a reducing agent is able to reduce quinone, drop oxygen content in the medium [12], thereby reducing the likelihood of

browning. The results of the study indicated that POD activity was strongly suppressed by L-cys and AA (Figure 4). In addition, L-cys and AA as natural nutrients is better anti-browning agent and have high safety, so can be used as inhibitors to control the browning of fresh-cut potato processing.

4. Conclusion

In summary, the optimal pH and temperature of POD was 6.0 and 55°C respectively. So the storage and processing of fresh-cut potato should be avoided at pH 6.0 and 55°C. L-cys and AA had good inhibitory effect on the activity of POD in potato.

Acknowledgments

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