

Enzymatic properties on browning of fresh-cut potato

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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 content of polyphenols and enzymatic properties in potato. In this study, it was shown that the optimal pH and temperature of polyphenol oxidase (PPO) was 6.5 and 40°C. The 0.02% sodium hydrogen sulfite (NaHSO₃), L-cysteine (L-cys) and ascorbic acid had good inhibitory effect on PPO activity. The results will provide the theoretical basis and practical guidance for the browning inhibition of fresh-cut potato.

1. Introduction

Studies have shown that enzymatic browning is observed upon air exposure [1], closely related to phenolic substances content and composition, and associated with polyphenol oxidase (PPO) [2-4], peroxidase (POD) and other enzymes [5, 6]. The browning of strawberry fruit seemed to be in relation to both oxidase activities [5]. Jin et al. [7] found that peach fruit browning was caused by the combination of PPO, POD and phenylalanine ammonialyase (PAL). The enzymatic reaction of PPO is one of the major reasons of browning and quality deterioration during fruits and vegetables processing and storage. It was found that the activity of enzymatic browning-related enzymes in different varieties of pear was different [8]. Graham-Acquaah et al. [6] showed that PPO activity of fresh-cut artichoke showed an increasing trend during storage, and the increase of PPO activity was the most significant in Day 3, which was significantly higher than the initial value.

The above research shows that PPO is the main enzymes related to enzymatic browning of fruits and vegetables. Potato is a typical material easy browning, but there is no system research about potato PPO. Therefore, the composition and content of potato polyphenols were identified in the study. The reaction kinetics of potato PPO 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 PPO, the effects of different substrate concentration and inhibitors on PPO activity.



2.2. Effects of pH on PPO 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 PPO activity. The potato PPO activity under different pH conditions was determined as follows.

Taken 2 mL 0.2 mol/L pH 5.5 acetic acid-sodium acetate buffer solutions, added 1mL 0.05 mol/L catechol as substrate, added 0.1mL enzyme solution, rapidly shaken and kept the reaction system for 10 min. The reaction system was scanned between 330 and 490 nm, distilled water instead of substrate was used as control for baseline scan. Taken the above reaction system, added enzyme and measured absorption value in the maximum absorption wavelength of product, and recorded every 15s. The absorbance of the enzymatic reaction system per gram per minute was increased by one as one PPO activity unit (U). The result was expressed as $1\text{ U} = \Delta\text{OD}_{\lambda\text{max}}/(\text{min.g m}_t)$.

2.3. Effects of Temperature on PPO Activity

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

2.4. Effects of Substrate Concentration on PPO Activity

The different concentrations (0, 4, 8, 12, 16, 20, 30, 40, 60, 80 and 100 mmol/L) of the catechol, chlorogenic acid, caffeic acid were substrates. The PPO activity was separately measured in each substrate on the maximum 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 PPO 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 PPO activity. The results were expressed relative enzyme activity. The formulas were as the following.

Relative enzyme activity (%) = Residual PPO activity / PPO 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 PPO Activity in Potato

As shown in Figure 1, the change of pH had a great effect on PPO activity of potato. There were two peaks of PPO activity in the range of pH 2.0-9.0. The maximum PPO activity was 9.771U when pH was 6.5, and there was a shoulder peak at pH 4.0. In pH 2.0-4.0 and 4.5-6.5 range, the PPO activity of potato significantly increased with the increase of pH ($p < 0.05$). From pH 7.5 to 9.0, PPO activity significantly decreased ($p < 0.05$) with the increase of pH. When pH was 9.0, the activity of PPO was completely lost, while when pH was 2.0, the activity of PPO was only 46.10 % for the optimum pH. So the optimum pH of PPO in potato was 6.5, similar to that of plums (6.0) [9] and mauritia flexuosa (7.0) [10].

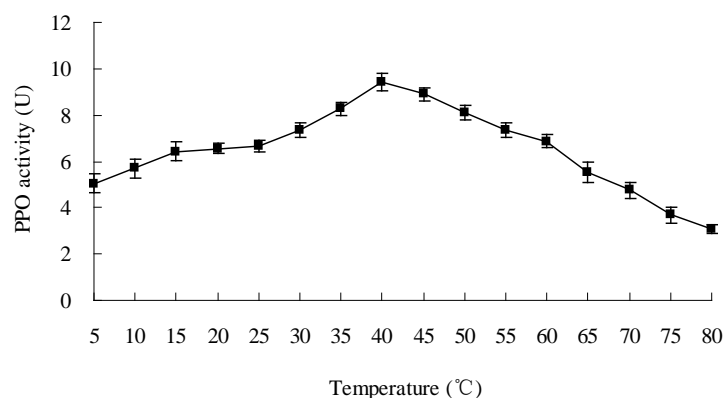


Figure 1. Effects of pH on PPO activity in potato

3.2. Effects of Temperature on PPO Activity in Potato

The activity of PPO increased with the increase of temperature, and the highest enzyme activity reached 9.423U at 40°C, but when the temperature continued to rise, the activity of enzyme showed a downward trend (Figure 2).

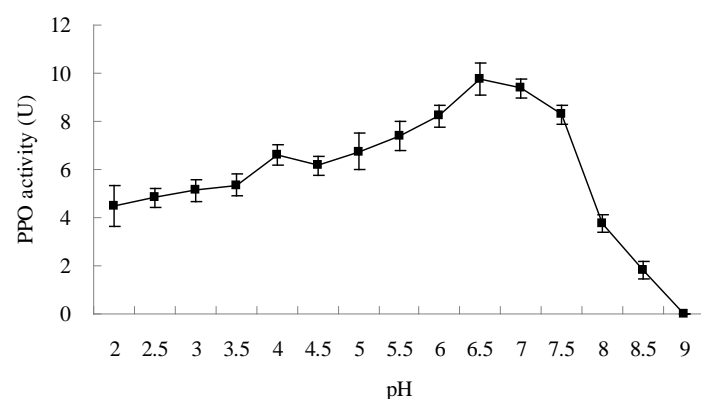


Figure 2. Effects of temperature on PPO activity in potato

PPO activity at 5°C was 53.53% at 40°C, and PPO activity at 80°C was 32.70% at 40°C. Therefore, the optimum temperature of PPO in potato was 40°C. The optimum temperature for PPO from Iranian medlar fruit was 35°C [11]. The optimum temperature values of PPO extracted from plums was 25°C [9]. It can be seen that the optimum temperature of PPO in different species is very different. In 5-15°C and 25-40°C range, with the increase of temperature, the PPO activity of potato significantly increased ($p < 0.05$). From 40 to 80°C, PPO activity was significantly decreased ($p < 0.05$) with temperature increased. Temperature had a great influence on PPO activity, due to the high temperature would destroy the enzyme protein which led to inactivation. Therefore, the effect of temperature was more significant at higher temperature.

3.3. Effects of Substrate Concentration on PPO Activity in Potato

The effect of substrate concentration of different phenols on PPO activity in potato was dramatically different (Figure 3). The activity of PPO in potato was the highest when chlorogenic acid was used as substrate. When the concentration of chlorogenic acid was 12-100mmol/L, the activity of PPO was dramatically higher than that of caffeic acid and catechol ($p < 0.05$). When the concentration of chlorogenic acid was 0-12mmol/L, PPO activity increased linearly with concentration increase, while PPO activity was raised slowly with 12-20mmol/L. When the concentration was greater than 20mmol/L, the change of PPO activity was not significant. Caffeic acid as substrate, the variation of

PPO activity with the increase of substrate concentration was similar to chlorogenic acid. Catechol as substrate, when the concentration was 12-100mmol/L, the activity of PPO was dramatically less than other phenolic substrates ($p < 0.05$). When the concentration was lower than 40mmol/L, the activity of PPO increased linearly with the increase of substrate concentration, and the activity of PPO increased slowly when the concentration was greater than 40mmol/L. The above results demonstrated that PPO activity in potato was not only affected by phenolic species, associated with corresponding substrate concentration. The kinetics of enzymatic browning reaction catalyzed by PPO was followed the Michaelis-Menten equation, which was consistent with PPO in strawberry [12].

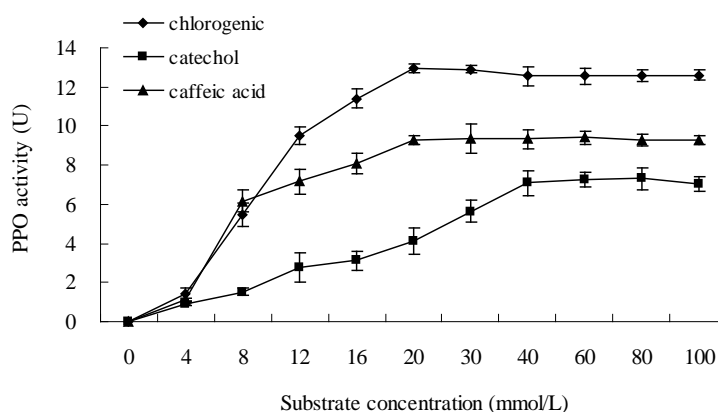


Figure 3. Effects of substrate concentration on PPO activity in potato

The K_m represents the corresponding substrate concentration when the reaction rate reaches half of the maximum reaction rate, and when K_m is smaller, affinity between enzyme and substrate is bigger [13]. It can be seen from Figure 3 that the order of binding ability of PPO to each substrate was chlorogenic acid > caffeic acid > catechol, and chlorogenic acid was the most suitable substrate. The result was different from that the optimum substrate of *artemisia selengensis* PPO [14] and loquat PPO [15] were catechol. The phenolic substances of potato were chlorogenic acid and catechins. The optimal substrate of PPO was chlorogenic acid. It was deduced that the reaction of PPO catalytic chlorogenic acid is the main cause of fresh-cut potato browning.

3.4. Effects of Inhibitors on PPO Activity in Potato

It was indicated that PPO activity was inhibited by different degrees of inhibition effects (Figure 4). In the same concentration range, the inhibitory effects of different inhibitors on PPO were different. EDTA had a certain inhibitory effect on PPO activity of potato. PPO activity of potato treated with 0.02 % EDTA was 67.782 % of the untreated. But with the increase of concentration, the inhibitory effect was not obvious. Potato processing with 0.02 % NaCl, PPO activity was 61.726 % of the untreated. CA had a certain inhibitory effect on PPO activity of potato. 0.02 % CA could significantly reduce the PPO activity ($p < 0.05$), with the increase of concentration, the inhibitory effect of PPO was not significant. NaHSO_3 , L-cys and AA had good inhibitory effect on PPO activity in potato, when the concentration was only 0.02 %, the activity of PPO could be inhibited completely. NaHSO_3 could inhibit the activity of potato PPO. But because of the sulfite and its decomposition product SO_2 on the health of the human body is harmful, NaHSO_3 used in food obtains strict restrictions. The results of the study indicated that PPO activity was strongly suppressed by L-cys and AA. In addition, L-cys and AA as natural nutrients are better anti-browning agent and have high safety, so can be used as inhibitors to control the browning of fresh-cut potato processing.

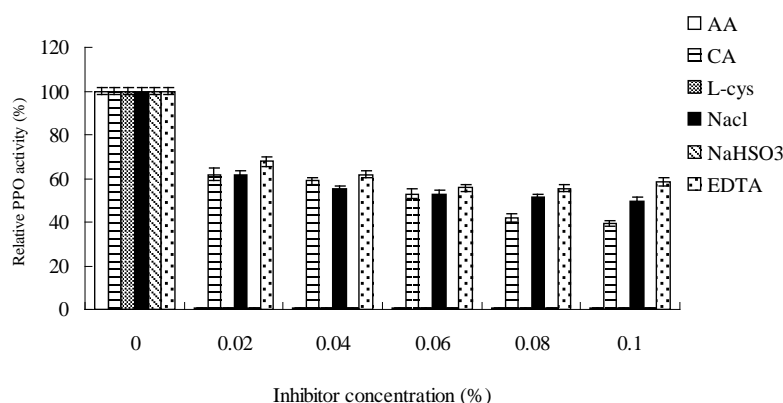


Figure 4. Effects of inhibitors on PPO activity in potato

4. Conclusion

In summary, the optimal pH and temperature of PPO was respectively 6.5 and 40°C. So the storage and processing of fresh-cut potato should be avoided at pH 6.5 and 40°C. L-cys and AA had good inhibitory effect on the activity of PPO in potato.

Acknowledgments

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