Properties and Extraction of Crude Pectin Esterase from Potato (Solanum Tuberosum)

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Abstract. In order to study the enzymatic properties of potato esterase, the crude enzyme of potato esterase was extracted with phosphate buffer solution, and the extraction conditions were optimized. The optimal condition is: the optimal condition for phosphate buffer solution pH 7.0, water bath time for 30 min, extraction temperature 35°C, and solid-liquid ratio 1:4 (g/mL). The property analysis showed that the potato esterase extracted from phosphate buffer solution was not a single esterase, but a kind of esterase isozyme. At 273nm and 325nm, the potato esterase crude enzyme had two characteristics uv absorption peak, its isoelectric point was near pI 4.4, and its molecular weight was roughly distributed at 16.4, 20.866, 22.73 and 40.286 KDa. In 40°C ~ 45°C, under the condition of pH 7.0, better able to maintain its activity, and seven kinds of metal ions are inhibitory effect to it. This study provides a theoretical basis for further understanding the enzymatic characteristics of potato esterase and its application in pesticide residue detection.

Keywords: solanum tuberosum; esterase; extraction conditions; properties.

1. Introduction

Esterase is a general term for catalyzing the hydrolysis of ester bonds and synthetases, which is widely found in animals, plants and microorganisms. Most esterases are encoded by multiple gene families, and there are differences in esterase isoenzymes between species and subspecies, so they are important target enzymes for evolutionary relationship analysis and cultivar identification [1]. Organophosphorus insecticides can form covalent bonds with serine residues at the active site of human acetylcholinesterase (AChE)[2], inactivating acetylcholinesterase, and inhibiting normal nerve conduction. Thus causes the entire physiological and biochemical process maladjustment, produces the toxicity to the organism[3]. Based on this toxicity mechanism, a method for detecting organophosphorus pesticide residues by acetylcholinesterase inhibition method has emerged, such as test paper method[4], electrochemical method[5] and so on. This method is different from chromatography and mass spectrometry relying on large analytical instruments[6]. Using the strong affinity of pesticides and acetylcholinesterase, it can quickly and sensitively detect pesticide residues. The commonly used AChE is extracted from animal and insect tissues or blood. The extraction process requires complex purification techniques and low temperature treatment[7], which means that the widespread use of this enzyme in rapid detection is limited. Relatively speaking, plant esterase is economically easy to obtain, extract and preserve, which has a broad application prospect. The extracted esterase activity is different, and the affinity for pesticides will be different. Therefore, finding a suitable plant esterase source is important for detecting pesticide residues by enzyme inhibition.

We tried to extract plant esterase from fresh potato tubers, and explored the relevant properties of the
crude enzyme before purifying the enzyme. This research would provide a reference for further comparative analysis of enzymatic properties of purified esterases and further search for the general rule of potato esterase activity and its foundation in the application of pesticide residue detection.

2. Materials and Methods

2.1. Materials and Instruments

Potatoes were acquired from Dingxi city (Gansu, China), and potato cultivar is Qingshu9. α-naphthyl acetate, α-naphthol and Fast blue B salt were purchased from Shanghai Yuanye Biotechnology Co., Ltd. (Shanghai, China). Disodium hydrogen phosphate, sodium dihydrogen phosphate, CaCl₂, BaCl₂, MgCl₂, AlCl₃, CuSO₄, FeSO₄, MnSO₄ and citric acid were purchased from Chengdu Kelong Chemical Reagent Factory (Chengdu, China). All of these reagents were analytical grade and used as received. UV-vis spectra were recorded using a UV-3100PC type spectrophotometer, which purchased from Shanghai Mepuda Instrument Co., Ltd. (Shanghai, China). The gel imaging using a Gel Doc2000 gel imaging system (Bio-Rad, USA).

2.2. Analytical Methods

2.2.1. Method for Extracting Potato Esterase Crude Enzyme. Wash the potatoes and peel them then broken with a broken machine. Weigh 5g and add 25 mL of phosphate buffer solution (PBS, 0.2mol/L, pH 6.5), and let it stand for 30 minutes in a water bath at 30°C, then filtered with gauze. The filtrate was centrifuged at 4000 r/min and 4°C for 15 min. The supernatant was taken as a crude enzyme solution and stored at 4 °C for use.

2.2.2. Determination of Potato Esterase Activity. Refer to the method of Ye et al. [8] with slightly modify. Prepare 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1 mmol/L α-naphthol standard solution to obtain a linear regression equation between α-naphthol concentration (Y) and absorbance (X) as Y=2.1144X - 0.0388 (R² = 0.999). The linear relationship of the regression equation is good which can be used to test the determination of esterase activity. The unit enzyme activity is calculated by the follow formula:

\[ E_{As} = \frac{k \times OD + b}{5 \times V} \times N \]

Where \( E_{As} \)--unit enzyme activity of the enzyme solution, U/mL (1 U is defined as the amount of enzyme required to catalyze 1 μmol of α-naphthol per minute under certain conditions); \( N \)--dilution of the enzyme solution; \( k \)--the slope of the alpha-naphthol standard curve; \( b \)--the intercept of the alpha-naphthol standard curve; \( OD \)--absorbance of the reaction solution; \( V \)--the volume of the reaction enzyme solution, mL; 5--the reaction time was determined to be 5 min.

2.2.3. Optimization of Extraction Conditions for Potato Esterase. (1) Single factor experimental design.

When the pH of the PBS is 6.5, the temperature is 30 °C, and the time is 20 min, the ratio of material to liquid (g/mL) is set to 1:3, 1:4, 1:5, 1: 6:1:7. When the pH is 6.5, the ratio of material to liquid is 1:5, and the temperature is 30 °C, the time is set at 10, 20, 30, 40, 50 min. When the temperature is 30 °C, the time is 40 min, and the ratio of material to liquid is 1:5, the pH is set to 5.5, 6.0, 6.5, 7.0, 7.5. When the pH is 6.5, the time is 20 min, and the ratio of material to liquid is 1:5, the temperature is set at 25, 30, 35, 40, 45 °C. Taking the unit enzyme activity as the evaluation index of the extraction effect. Analyzing the effect of the various factors on the extraction of potato esterase.

(2) Orthogonal design

According to the results of single factor test, the pH (A), extraction time (B), extraction temperature (C) and ratio of material to liquid (D) were selected as the investigation factors, and the unit enzyme activity of potato esterase was taken as the investigation index. L₉ (3⁴) orthogonal experiments were performed, each factor was paralleled 3 times, and the results were averaged.
2.2.4. Study on Properties of Potato Esterase. (1) Determination of ultraviolet absorption spectrum
Analyzed the peak shape and peak position of the ultraviolet absorption curve at 200~800 nm.
(2) Determination of isoelectric point of potato esterase
Adjust the crude enzyme solution pH to 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, 11.0, and let it stand
at 4°C for 30 min. Then centrifuge at 4000 r/min for 15 min at 4°C. Take the supernatant, determine
the protein content[9]. Then select 11 points (take a point every 0.4) near the lowest point of the
protein content, repeat the above steps to determine the isoelectric point of potato esterase.
(3) SDS-PAGE gel electrophoresis analysis
The method of SDS-PAGE gel electrophoresis were reference to Laemmli et al. [10]. The
electrophoresis conditions were: the concentration of upper gel is 4%, voltage of 90 V, 30 min;
separation gel concentration of 12%, voltage of 120 V, 120 min.
(4) Determination of optimum temperature of potato esterase
Take a certain amount of potato esterase crude enzyme solution in a 25, 30, 35, 40, 45, 50, 60 °C water
bath, respectively, after 15 min incubation, determine the unit enzyme activity of the crude enzyme
solution. The enzyme activity of the unheated crude enzyme solution is 100%. Calculate the relative
enzyme activity, and the optimum temperature of potato esterase was investigated.
(5) Determination of optimum pH of potato esterase
Take a certain amount of potato esterase crude enzyme solution, add 0.2 mol/L buffer solution with pH
values of 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0 respectively, and incubate in a 30°C water bath for 15 min.
Then measure the crude enzyme solution. The enzyme activity per unit enzyme was 100% of the
enzyme activity of the unadjusted pH crude enzyme solution, and the relative enzyme activity was
calculated to determine the optimum pH of the potato esterase.
(6) Effect of metal ions on potato esterase
Take appropriate amount of potato esterase crude enzyme solution, add 1 mL of 2, 6, 10 mmol/L Ca^{2+},
Ba^{2+}, Mg^{2+}, Al^{3+}, Cu^{2+}, Fe^{2+}, Mn^{2+} solution, respectively, and measure the unit enzyme activity. The
enzyme activity of the crude enzyme solution without adding metal ion solution was 100%, and the
relative enzyme activity was calculated.

2.3. Statistical Analysis
The experimental data was analyzed by IBM SPSS Statistics data editor and plotted with Origin Pro
9.0 software. Electropherogram acquisition and molecular weight calculation were performed using
Quantity one software.

3. Results and Analysis

3.1. Effects of Extraction Conditions on Crude Enzyme Activity of Potato Esterase
In Fig. 1a, the activity per unit of enzyme is continuously increased, reaching a maximum at 1:5
(g/mL). And then gradually reduced. When the optimum ratio of material to liquid is reached, the
esterase dissolution in the solution tends to be balanced, and too much solvent has a dilution effect on
the system, resulting in a decrease in unit enzyme activity. In Fig. 1b, the enzyme activity reached the
maximum at 20min, and the unit enzyme activity decreased gradually after 20min. When the
extraction time is short, the esterase is not easy to separate from other components [11], and the
enzyme activity is low. As the extraction time is extended, the esterase is fully dissolved and reaches
equilibrium at 20 min. Due to the conformational instability of the esterase in the solution state [12],
the extraction time is too long and the enzyme activity is likely to decrease. In Fig. 1c, the optimum
pH of the phosphate buffer solution is 6.5. At the same time, as shown in Fig. 1c, the effect of pH on
the enzyme is significant, and the unsuitable pH has a great influence on its stability. In Fig 1d, the
enzyme activity reached the maximum at 40 °C. The higher of temperature, the higher activity of
enzyme, which may be caused by the high temperature to increase the solubility of the enzyme protein,
but too high temperature will cause the hydrophobic group to be exposed, and the solubility will
decrease [13], resulting in lower esterase activity. At lower temperature, the enzyme is difficult to
release completely, resulting in lower enzyme activity.
Figure 1. Effect of extract conditions on enzyme activity: (a) material - liquid ratio, (b) water-bath time, (c) pH, (d) extraction temperature

3.2. Orthogonal Optimization Experiment of Crude Enzyme Extraction Conditions of Potato Esterase

The results of orthogonal enzyme experiment to optimize the extraction of potato esterase crude enzyme are shown in Table 1.

| No. | pH A | Time B (min) | Temperature C (°C) | Solid-liquid D | Enzyme activity (U/mL) |
|-----|------|--------------|--------------------|----------------|-----------------------|
| 1   | 6.0  | 10           | 35                 | 4              | 709.846               |
| 2   | 6.0  | 20           | 40                 | 5              | 646.696               |
| 3   | 6.0  | 30           | 45                 | 6              | 533.928               |
| 4   | 6.5  | 10           | 40                 | 6              | 741.985               |
| 5   | 6.5  | 20           | 45                 | 4              | 806.262               |
| 6   | 6.5  | 30           | 35                 | 5              | 860.391               |
| 7   | 7.0  | 10           | 45                 | 5              | 881.817               |
| 8   | 7.0  | 20           | 35                 | 6              | 860.391               |
| 9   | 7.0  | 30           | 40                 | 4              | 973.723               |
| K1  | 1890.469 | 2333.647   | 2430.628           | 2489.831       |
| K2  | 2408.638 | 2313.349   | 2362.403           | 2388.904       | 7015.038              |
| K3  | 2715.931 | 2368.042   | 2222.007           | 2136.303       |
| k1  | 630.156 | 777.882     | 810.209            | 829.944        |
| k2  | 802.879 | 771.116     | 787.468            | 796.301        |
| k3  | 905.310 | 789.347     | 740.669            | 712.101        |
| Range | 275.154 | 18.231      | 69.540             | 117.843        |
| Priority | A>D>C>B |
| The optimal level | A₃ | B₃ | C₁ | D₁ |
| Optimal combination | A₃B₃C₁D₁ |

The results of orthogonal experiment
It can be seen from Table 1 that the range of pH is the largest, and the second is the ratio of material to liquid, temperature and water bath time. The optimal combination of extraction conditions is: the pH was 7.0, the time was 30 min, the temperature was 35 °C, and the ratio of material to liquid was 1:4. The verification experiments were carried out according to the optimal extraction conditions (A3B3C1D1). The unit enzyme activities of the measured potato esterase were 1053.224 U/mL, 961.882 U/mL, and 958.442 U/mL, respectively. The results showed that the optimized extraction conditions were stable and could be used as the best extraction conditions for extracting potato esterase.

3.3. Properties of Potato Esterase

![Graphs](image)

Figure 2. Properties of crude pectin esterase from *Solanum tuberosum* (a UV- spectrometry, b pI analysis value, c SDS-PAGE, d temperature, e pH)

As shown in Fig. 2a, the crude enzyme solution of potato esterase has a major UV characteristic absorption peak at 325 nm and an insignificant absorption peak at 273 nm. In the near ultraviolet region (200~400 nm), aromatic amino acids have the ability to absorb light, and the maximum absorption peaks of tyrosine, phenylalanine and tryptophan are between 260 and 280 nm [14]. It is indicated that potato esterase is within the characteristic range of protein ultraviolet absorption spectrum.

When the isoelectric point of the protein is the same as the pH of the solution, the positive and negative charges generated by the dissociation of the protein molecules are equal, and at this time, the protein molecules will agglomerate and precipitate. Therefore, the protein has the least solubility at the isoelectric point and is most likely to form precipitates [15]. Fig. 2b shows that the protein content in the supernatant is the lowest when the pH is 4.4 (115.119 g/mL). It is concluded that the pI of potato esterase is about 4.4. At pH 2, probably due to the peracid environment leading to denaturation of the enzyme protein, thereby changing the conformation, affecting and resulting in lower content of
measured protein [16]. SDS-PAGE analysis showed (Fig. 2c) that there were 4 clear bands in the enzyme solution, indicating that the potato esterase extracted by phosphate buffer solution is not an enzyme, and its active component is esterase isoenzyme. Calculated the molecular weight distribution of potato esterase isoenzyme, and the results indicated that the molecular weight of potato esterase isoenzyme were 16.4, 20.866, 22.73, and 40.286 KDa, which is consistent with the previous report of most plant esterase molecular weights from 20kDa to 60kDa [17~18].

The relative enzyme activity of potato esterase tends to be stable when kept at 25~50 °C. The relative enzyme activities at 40°C and 45°C were 96.13% and 96.24%, respectively. It’s indicating the optimal temperature of potato esterase between 40~45 °C. When the temperature exceeds 50 °C, the enzyme activity begins to decrease sharply. It’s indicating that the excessive temperature has a destructive effect on the specific structure of the enzyme active center, which denatures the enzyme protein [19] and the activity is reduced. The relative enzyme activity of potato esterase was stable between pH 4~6. The relative enzyme activity at pH 7 was 98.11%, which was higher than other groups. The active center of the esterase is located in a canyon area, and the bottleneck of the canyon area is a constricted area. The environment of strong acid and alkali may cause the amino acid conformation of this area to change, thereby inhibiting the activity of the enzyme [20].

Some metal ions can act as activators or inhibitors of the enzyme. By changing the configuration of the enzyme protein, the active site of the enzyme is more suitable for binding to the substrate [21]. In Table 2, Ca<sup>2+</sup> and Ba<sup>2+</sup> have inhibitory effects on potato esterase activity. Low concentration (2 mmol/L) of Mg<sup>2+</sup>, Al<sup>3+</sup> and Fe<sup>2+</sup> on potato esterase activity was greater than that of the three metal ions (6 mmol/L, 10 mmol/L); while Cu<sup>2+</sup> and Mn<sup>2+</sup> inhibited potato esterase with increasing concentration.

### Table 2. Relative enzyme activity of potato esterase with different metal ions

| Metal ions | Metal ion concentration (mmol/L) |
|------------|----------------------------------|
|            | 0      | 2      | 6      | 10     |
| Ca<sup>2+</sup> | 100±5.66a | 64.47±2.83b | 66.85±0.18b | 71.73±1.42b |
| Ba<sup>2+</sup> | 100±5.66a | 52.96±8.85c | 66.35±1.59bc | 69.35±0.53b |
| Mg<sup>2+</sup> | 100±5.66a | 31.19±5.31c | 64.97±1.06b | 73.35±1.24b |
| Al<sup>3+</sup> | 100±5.66a | 47.58±0.18c | 77.86±1.95b | 78.98±0.35b |
| Cu<sup>2+</sup> | 100±5.66a | 44.20±2.12d | 58.71±2.83c | 73.60±1.59b |
| Fe<sup>2+</sup> | 100±5.66a | 39.20±4.60c | 59.96±7.08b | 86.49±2.48a |
| Mn<sup>2+</sup> | 100±5.66a | 47.45±0.71c | 53.71±1.42c | 89.24±4.60b |

Control: Relative activity of untreated potato esterase. Different letters following date at same line mean significant difference among treatments (P<0.05).

### 4. Conclusion

In this study, the optimal extraction conditions of potato esterase were obtained, and the properties of crude enzymes of fresh potato tuber esterase were preliminarily determined. It was found that its active component consisted of potato esterase isoenzyme, rather than a single esterase. The optimal temperature of potato esterase was in the range of 40~45 °C. The optimum pH is 7, and several metal ions selected in the experiment have inhibitory effects on potato esterase. The enzyme solution used in this study is a crude enzyme that has not been purified, and its related properties will be different in the enzymatic properties after purification. The results of this study will guide the targeted purification of the ester. The enzyme provides more reference, which can provide a theoretical basis for further in-depth research and application.

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