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Thermal Inactivation and Kinetic Characterisation of Peroxidase from Leaves of *Brassica chinensis* L.

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Abstract: As one of the perennial vegetable varieties grown in the south of Yangtze River in China, *Brassica chinensis* L. can be produced and supplied to the market all year round and has high economic value. However, there is little direct literature on postharvest heat treatment of *Brassica chinensis* L. In this study, Peroxidase (POD) was extracted from *Brassica chinensis* L. leaves and its enzymatic characteristics such as optimal pH, optimal temperature, catalytic activation energy, Km and Vmax, thermal stability and thermal denaturation activation energy were discussed. The results showed that with o-phenylenediamine as the hydrogen donor substrate of POD and hydrogen peroxide as the peroxide substrate, the optimal pH was 6.8, the optimal reaction temperature was 40°C and the activation energy of the catalytic reaction was 35.34 kJ/mol for POD in *Brassica chinensis* L. leaves. As a dual substrate reaction, when o-phenylenediamine was used as the substrate, the Km value of POD was 1.143 mmoL/L and Vmax was 142.86 U. When hydrogen peroxide was used as the substrate, the Km value of POD was 2.778 mmoL/L and Vmax was 111.11 U. Thermal inactivation studies showed first-order inactivation kinetic characteristics and the Arrhenius plot yielded a straight line with a slope equivalent to the thermal denaturation activation energy of POD at 199.54 kJ/moL. The thermal inactivation rate of POD varied significantly between 70 and 80°C (the rate constant k ranged from -0.446 to -0.970), indicating that the thermal denaturation of POD may occur between 70 and 80°C. It is expected that the above data would be beneficial to the optimization of quality control parameters in postharvest heat treatment and cold-chain transportation of *Brassica chinensis* L.

Keywords: *Brassica chinensis* L., Peroxidase, Heat Inactivation, Kinetic Characteristics, Activation Energy

Introduction

Brassica chinensis L. known as Shanghai green, rape, green cabbage, and baierbai, etc., belongs to the *Brassica*e family. *Brassica chinensis* L. is heat-resistant, cold-resistant, and insect-resistant and is a perennial cabbage variety planted in Shanghai and southern Jiangsu China. It has a crisp taste and natural taste and can be used in Japanese, Western, and Chinese dishes with high economic value. With the rapid development of logistics storage and distribution technology in China, the harvested fresh *Brassica chinensis* L. can be directly sold locally or transported to the north of China to make up for the shortage of vegetables in winter in the north. In recent years, with the expansion of the planting area of *Brassica chinensis* L. in southern Jiangsu, after blanching, the newly harvested *Brassica chinensis* L. are transported and exported through the cold chain to supermarkets in neighboring countries such as Japan.

Peroxidase (POD, EC 1.11.1.7) is widely distributed in nature and is a highly active redox enzyme in animals, plants, and microorganisms (Rusdi *et al*., 2014; Agunbiade *et al*., 2021; Zitare *et al*., 2021; Benslama *et al*., 2022). Blanching is an important step in the processing of fruits and vegetables. Fruits and vegetables can be heated to obtain storage stability, passivate enzymes that have adverse effects on product quality, reduce the number of microorganisms, remove oxygen from tissues, and enhance the color of most fruits and vegetables. Because POD is very heat-resistant, it can be used as a standard to

evaluate the adequacy of blanching of fruits and vegetables by analyzing POD activity before and after blanching (Adams *et al*., 2003).

At present, there is a lot of research on POD including separation (Dahdouh *et al*., 2020; 2021), purification (Al-madhagi *et al*., 2023; Oztekin *et al*., 2022), characterization (Dahdouh *et al*., 2022; Catucci *et al*., 2020), kinetic (Połata *et al*., 2009; Almaz and Agircelik, 2023) and application (Klanovicz *et al*., 2020; Altahir *et al*., 2020; Zhang *et al*., 2022; Ponce *et al*., 2004). However, there is no related literature on the POD of *Brassica chinensis* L. and heat treatment. In order to meet the needs of cold chain logistics and ensure the quality of *Brassica chinensis* L., it is urgent to understand some enzymatic characteristics of POD, especially the effect of heat treatment on POD activity and related kinetics of POD from leaves of *Brassica chinensis* L.

In this study, POD was extracted from leaves of *Brassica chinensis* L., and enzymatic characteristics such as optimum pH, optimum temperature, activation energy of catalytic reaction, Km and Vmax of corresponding test substrate, thermal stability and thermal denaturation activation energy were investigated, which may lay a theoretical foundation for the quality control of *Brassica chinensis* L. in the marketing link.

Materials and Methods

Materials and Chemicals

Brassica chinensis L. was purchased from Changshu City, Jiangsu Province of China. Disodium hydrogen phosphate, sodium dihydrogen phosphate, potassium dihydrogen phosphate, sodium chloride, ammonium sulfate, o-phenylenediamine, hydrogen peroxide, ethanol, etc., were purchased from Jiangsu Changsheng Chemical Co., LTD.

Extraction of POD from Leaves of Brassica chinensis L.

Extraction of POD from leaves of *Brassica chinensis* L. was based on the reported method (Serrano-Martínez *et al*., 2008) with a slight modification.100 g fresh leaves of *Brassica chinensis* L. were cut and added with 200 mL precooled phosphate buffer (0.05 moL/L, pH 7.0), homogenized and filtered with multi-layer gauze. The obtained homogenized pulp was centrifuged at 8000 r/min for 20 min. The supernatant was added with ammonium sulfate to 55% saturation and salted out for 2 h. Then the supernatant was centrifuged at 8000 r/min for 20 min to obtain the precipitate. Then the precipitate was dialyzed for 24 h to obtain a crude POD solution. The above operations were carried out at ±4°C.

Determination of POD Activity

POD activity from leaves of *Brassica chinensis* L. was determined based on the previous method (Fornera and Walde, 2010). 3 mL phosphate buffer (0.2 moL/L, pH 6.3), 0.2 mL o-phenylenediamine-ethanol solution (1%, w/w) and 0.5 mL hydrogen peroxide solution (0.3%, w/w) were added into the colorimetric dish. Finally, 0.5 mL crude POD solution was added. After mixing, the Optical Density (OD) change within 2 min at 600 nm was recorded. The OD-time curve was recorded with the OD value as the vertical coordinate and the reaction time as the horizontal coordinate. The POD activity was calculated from the slope of the initial straight-line part of the curve. An increase of OD value of 0.01 per minute was defined as an enzyme activity Unit (U). The blank control was phosphate buffer without POD (containing substrate o-phenylenediamine and hydrogen peroxide solution). The above operations were carried out at 4°C.

Determination of the Optimal Reaction pH of POD

At the optimum reaction temperature, 3 mL phosphate buffer (0.2 moL/L) at different pH (5.8, 6.0, 6.3, 6.5, 6.8, 7.0, 7.3, 7.5, and 8.0) and 0.5 mL crude POD solution were added into the colorimetric dish. The mixture was held for 10 min, respectively. Finally, 0.2 mL o-phenylenediamine ethanol solution (1%, w/w) and 0.5 mL hydrogen peroxide solution (0.3%, w/w) were added and the OD change within 2 min at 600 nm was recorded after mixing. The blank control was phosphate buffer with the corresponding pH and without POD (containing substrate o-phenylenediamine and hydrogen peroxide solution).

Determination of Optimal Reaction Temperature of POD

6 mL crude POD solution was taken in a test tube and placed in a constant temperature water bath at different temperatures. 3 mL phosphate buffer (0.2 moL/L, pH 6.3), 0.2 mL o-phenylenediamine ethanol solution (1%, w/w) and 0.5 mL hydrogen peroxide solution (0.3%, w/w) were added into the colorimetric dish. The colorimetric dish was placed on a UV-6100 UV-visible spectrophotometer and the temperature was equilibrated. 0.1 mL crude POD solution with the corresponding temperature was added. The change of OD within 2 min at 600 nm was recorded after mixing. The temperature was 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85 and 90°C, respectively.

Analysis of Activation Energy of POD Catalytic Reaction

3 mL phosphate buffer (0.2 moL/L, pH6.3), 0.2 mL o-phenylenediamine ethanol solution (1%, w/w) and 0.5 mL hydrogen peroxide (0.3%, w/w) solution were added into the colorimetric dish. The colorimetric dish was placed on a UV-6100 UV-visible spectrophotometer. After the temperature was balanced, 0.1 mL crude POD solution was added. The change of OD value within 3 min at 600 nm was recorded after mixing. The temperature was 20, 25, 30, 35, 40, 45, 50, 55 and 60°C respectively. The OD-time

curve was recorded with OD value as the vertical coordinate and reaction time as the horizontal coordinate. The velocity constant k of enzyme-catalyzed reaction at different temperatures was calculated from the slope of the initial straight line part of the curve. The logarithm lg of the velocity constant k of the enzyme-catalyzed reaction was used to plot 1/T and a straight line was obtained. According to the Arrnenius equation k = Ae Ea/RT, the slope of the line was Ea/2.3 R, where Ea was the activation energy of the enzyme-catalyzed reaction and R was the universal gas constant (Wang *et al*., 2023).

Determination of Km and Vmax of POD with o-Phenylenediamine as Substrate

At the optimum reaction temperature, 3 mL phosphate buffer (0.2 moL/L, pH6.3), 0.2 mL o-phenylenediamine ethanol solution with different concentrations (0.4, 0.6, 0.8, 1.0, 1.2 and 1.6%, w/w) and 0.5 mL hydrogen peroxide solution (0.3%, w/w) were added into the colorimetric dish. Then 0.1mL POD solution was added and mixed. The change of OD value within 2 min at 600 nm was recorded. The blank control was phosphate buffer with the corresponding pH and without POD (containing 0.2 mL o-phenylenediamine-ethanol solution with the corresponding concentration and 0.5 mL hydrogen peroxide solution (0.3%, w/w). The OD-time curve was recorded with the OD value as the vertical coordinate and the reaction time as the horizontal coordinate. The initial reaction rate V was calculated from the slope of the first straight part of the curve (an increase of OD value 0.01 per minute was defined as an enzyme activity unit). By using the double reciprocal plotting method (Lineweaver-Burk method) (Basumatary *et al*., 2023), a straight line was obtained by plotting 1/V against 1/[S]. Its slope was Km/Vmax and its intercept on the vertical axis was 1/Vmax. Vmax can be obtained. The intercept on the horizontal axis was -1/Km and the Km value can be obtained.

Determination of Km and Vmax of POD with Hydrogen Peroxide as Substrate

At the optimal reaction temperature, 3 mL phosphate buffer (0.2 moL/L, pH6.3), 0.2 mL o-phenylenediamine ethanol solution (1%, w/w), 0.5 mL hydrogen peroxide solution with different concentrations (0.1, 0.2, 0.3, 0.4, 0.5 and 0.6%, w/w) were added into the colorimetric dish. Then 0.1 mL POD solution was added to the colorimetric dish. After mixing, the change of OD value within 2 min at 600 nm was recorded. The blank control was phosphate buffer with the corresponding pH and without POD (containing 0.2 mL o-phenylenediamine-ethanol solution (1%, w/w) and 0.5 mL hydrogen peroxide solution). The OD time curve was recorded with the OD value as the vertical coordinate and the reaction time as the horizontal coordinate. The initial reaction rate V was calculated from the slope of the initial straight part of the curve. By using the double reciprocal plotting method (Lineweaver-Burk method) (Basumatary *et al*., 2023), a straight line was obtained by plotting 1/V against 1/[S]. Its slope was Km/Vmax and its intercept on the vertical axis was 1/Vmax.Vmax can be obtained. The intercept on the horizontal axis was -1/Km and the Km value can be obtained.

Analysis of Thermal Stability of POD

6 mL crude POD solution was added into the test tube and incubated in a water bath at 40°C for 1 minute. After incubation, the crude POD solution was moved to another water bath at 100°C. Then, 0.2 mL crude POD solution was removed from the test tube at 0-30 s, 1, 1.5, 2, 3, 5, 7, and 10 min respectively, and cooled to 0°C in an ice bath immediately. The activity of the crude POD solution kept at 0°C without heat treatment was taken as 1 and the relative residual activity of the enzyme solution after different heat treatment was calculated.

Analysis of Activation Energy of Thermal Denaturation of POD

6 mL crude POD solution was added into the test tube and incubated in a water bath at 20, 25, 30, 35, 40, 45, 50, 55, 60, 70, 80, 90, and 100°C, respectively. Then the POD solution (0.2 mL) was removed from the test tube and cooled to 0°C at 0-30 s, 1, 1.5, 2, 2.5, 3, 5, 7, and 10 min, respectively. The activity of the crude POD solution without heat treatment kept at 0°C was taken as 1. The relative residual activity at different temperatures and different holding times was calculated. The logarithm of the residual activity percentage of POD was plotted against the holding time. The slope k of the straight line was the denaturation rate constant of the enzyme. Logarithm ln of the enzyme thermal inactivation rate constant k (absolute value) is plotted against 1/T (K degree temperature) to obtain a straight line. According to the Arrnenius equation k = Ae-Ea/RT, the slope of the line was -Ea/2.3R, where Ea was the activation energy of enzyme thermal denaturation. R was the universal gas constant (8.314J /(moL*K)) (Wang *et al*., 2023).

Data Processing

All experiments were performed in triplicate and the data were subjected to a one-way analysis of variance using Origin 2016 (OriginLab Corporation, Northampton, MA, USA). The results were expressed as mean ± standard deviation and the statistical significance was set at $p<0.05$.

Results and Discussion

The Optimal Reaction pH of POD

Enzyme activity is largely affected by pH, so it is important to maintain a certain pH value in the process of

enzyme activity determination (Damodaran and Parkin, 2017). The effect of pH on the activity of POD from leaves of *Brassica chinensis* L. was shown in Fig. 1. It could be seen that the enzyme activity was highest at pH 6.8. The optimal pH of enzymes in plants and microorganisms is mostly around 4.5-6.5 (Almaz and Agircelik, 2023). The reasons why pH affects enzyme activity may include the following aspects. First, excessive acid or alkali destroys the spatial structure of the enzyme, resulting in the denaturation of the enzyme, the change of enzyme conformation, and the decrease or loss of enzyme activity. Second, pH affects the dissociation state of the substrate or the dissociation state of the relevant groups on the active site of the enzyme molecule or the dissociation state of the enzyme-substrate complex. The substrate cannot combine with the enzyme to form the enzyme-substrate complex, or the product cannot be generated after the formation of the enzyme-substrate complex, which reduces the enzyme activity. Third, pH affects the dissociation of groups related to the maintenance of the spatial structure of the enzyme molecules, thus changing the conformation of the active site of the enzyme and reducing the activity of the enzyme (Whitaker *et al*., 2002).

The Optimum Reaction Temperature of POD

The speed of enzymatic chemical reaction is closely related to temperature. The influence of temperature on the speed of enzymatic chemical reactions is mainly manifested in two aspects. When the temperature rises, the reaction speed is accelerated, just like the general chemical reaction. With the gradual increase in temperature, the enzyme will be deactivated due to gradual denaturation (Damodaran and Parkin, 2017). The effect of temperature on the activity of POD from leaves of *Brassica chinensis* L. was shown in Fig. 2. Usually, in a certain temperature range, the enzyme activity increases with the increase of temperature and decreases when it exceeds a certain temperature limit. The temperature of the highest activity is the optimum reaction temperature of the enzyme (Połata *et al*., 2009). It could be seen from Fig. 2 that the activity of POD was the highest at 40°C, indicating that the optimal reaction temperature of POD was 40°C.

There are great differences in the optimum temperature of peroxidase from different sources. Usually, the optimum temperature of plant peroxidase activity is 15-70°C (De Oliveira *et al*., 2021). The optimum temperature of peroxidase activity in peaches was 40°C (Neves, 2002). The optimum temperature of peroxidase extracted from Litchi pericarp was 70°C (Mizobutsi *et al*., 2010).

Activation Energy of Catalytic Reaction of POD

The effect of temperature on the formation rate of products catalyzed by POD was presented in Fig. 3. The velocity constant k of POD catalytic reaction at different temperatures was calculated from the slope of the initial straight line part of the curve. A straight line was obtained by plotting 1/T with the logarithm lg of the speed constant K of the enzyme-catalyzed reaction (Fig. 4). According to the Arrnenius equation k = Ae Ea/RT, the slope of the straight line was Ea/2.3R, where Ea was the activation energy of the enzyme-catalyzed reaction (Wang *et al*., 2023). As can be seen from Fig. 4, the slope of the straight line was -1848. The activation energy Ea of POD catalyzed reaction was calculated to be 35.34 kJ/moL. The lower activation energy of POD reveals the nature of the damage and easy browning of *Brassica chinensis* L.

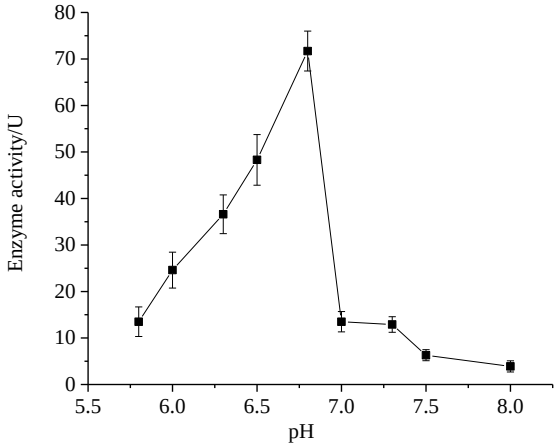

Fig. 1: Effect of pH on the activity of POD from leaves of *Brassica chinensis* L.

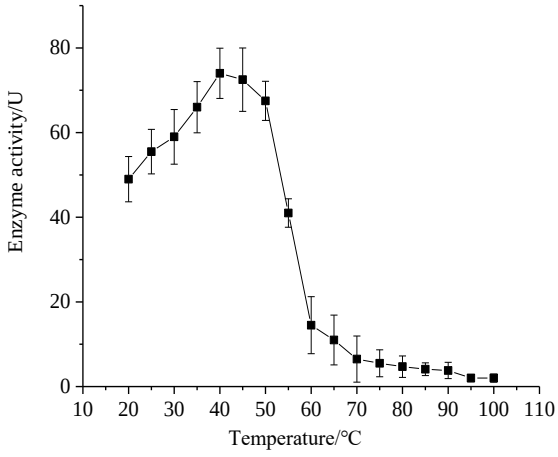

Fig. 2: Effect of temperature on activity of POD from leaves of *Brassica chinensis* L.

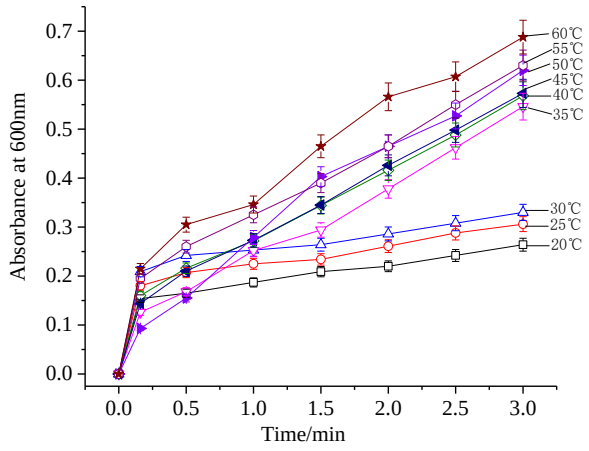

Fig. 3: Effect of temperature on the rate of product formation in the POD catalytic reaction

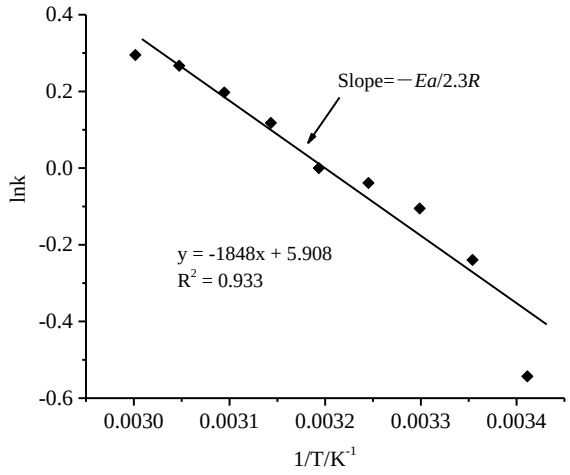

Fig. 4: Effect of temperature on the rate constant of POD catalytic reaction

In addition, it can be seen from Fig. 4 that the speed constant of the POD catalytic reaction increased steadily in the temperature range of 20-60°C. According to the later POD thermal stability analysis, the denaturation temperature of the POD may be between 70 and 80°C (Fig. 9). Therefore, when the temperature increased before peroxidase denaturation, the speed of POD catalytic reaction was accelerated, indicating that low-temperature cold storage and hot blanching technology were the first choice for fruit and vegetable food preservation (Benslama *et al*., 2022; Połata *et al*., 2009).

Km and Vmax of POD with o-Phenylenediamine as Substrate

The Km value is the substrate concentration when the initial reaction speed V is half of the maximum reaction speed Vmax. Km is a very important constant in enzyme research and one of the characteristic constants of enzymes. It is generally only related to the properties of enzymes and has nothing to do with the concentration of enzymes. If an enzyme has several substrates, each substrate corresponds to a specific Km value (Damodaran and Parkin, 2017). Therefore, for interested enzymes, the determination of Vmax and Km is extremely important. The determination of these two terms can enable us to predict the speed of enzymatic catalytic reaction under a certain concentration of enzyme and substrate.

There are usually two types of hydrogen donor substrates for peroxidase. One is a phenolic compound and the other is an amine compound. Guaiacol is a frequently used hydrogen donor substrate (Whitaker *et al*., 2002). However, the preliminary experiment showed that o-phenylenediamine was more sensitive than guaiacol when the peroxidase activity and residual peroxidase activity of vegetables were determined by spectrophotometry. Therefore, o-phenylenediamine was used as the hydrogen donor substrate of peroxidase in this experiment.

In view of the fact that in this experiment, the reaction catalyzed by POD involved two substrates and the corresponding Km of each substrate was different from that of Vmax. Under the optimum reaction conditions, using the double reciprocal mapping method (Fig. 5), POD was used to catalyze the reaction of o-phenylenediamine with hydrogen peroxide at different concentrations (1.0-4.0 mmoL/L). The activity of POD was determined and the *Km* value of the enzyme was 1.143 mmoL and 142.86 U.

According to the Michaelis equation derived by Michaelis and Menten in 1913 (Damodaran and Parkin, 2017), an approximate km value can be obtained by arranging two measurements. The difference between the two measurements is that the substrate concentration used in one of the two tests is 5-20 times that of the other and the highest substrate concentration used in the determination should not reach the level of complete saturation of the enzyme. The measured substrate concentrations $[S]_1$, $[S]_2$ and their corresponding initial reaction velocities V_1 and V_2 are brought into the derived formula:

$$Km = \frac{[S]_1[S]_2(V_1-V_2)}{V_2[S]_1-V_1[S]_2} \quad (1)$$

The *Km* can be calculated. The greater the difference between the two substrate concentrations $[S]_1$ and $[S]_2$, the more accurate the measured *Km*.

POD was used to catalyze the reaction of o-phenylenediamine with hydrogen peroxide at different concentrations (1.0-4.0 mmoL/L) in Fig. 5. The initial reaction velocity corresponding to the lowest and highest substrate concentrations was put into Eq. (1) to calculate *Km*. The *Km* was 1.131 mmoL/L, which was very close to *Km* (1.143 mmoL/L) obtained by the double reciprocal

method in Fig. 5. The results indicated that the double reciprocal method was used to determine the Michaelis constant of POD (Fig. 5) and the concentration of substrate o-phenylenediamine was selected within a reasonable range.

Km and Vmax of POD with Hydrogen Peroxide as Substrate

In addition to the hydrogen donor substrate, the other substrate of peroxidase is peroxide. The peroxide base is mainly hydrogen peroxide. However, high concentrations of hydrogen peroxide can inactivate enzymes. The concentration of hydrogen peroxide affects the activity of catalase (Valderrama *et al*., 2002). Therefore, it is necessary to explore the hydrogen peroxide concentration of the substrate in the design of the experiment so that its selection should be within a reasonable range.

Under the optimal reaction conditions, POD was used to catalyze the reaction of hydrogen peroxide with o-phenylenediamine at different concentrations (2.0-13.0 mmoL/L) by double-reciprocal mapping method (Fig. 6). Activity of POD was measured. Km of the POD was obtained as 2.778 mmoL/L, and Vmax was 111.11 U.

The initial reaction velocity corresponding to the lowest and highest substrate concentration in Fig. 6 was brought into Eq. (1) to calculate Km. The Km was 2.671 mmoL/L, which was basically close to Km (2.778 mmoL/L) obtained by the double reciprocal method in Fig. 6. The results showed that the double reciprocal method was used to determine the Michaelis constant of POD (Fig. 6) and the concentration of hydrogen peroxide in the substrate was also selected within a reasonable range.

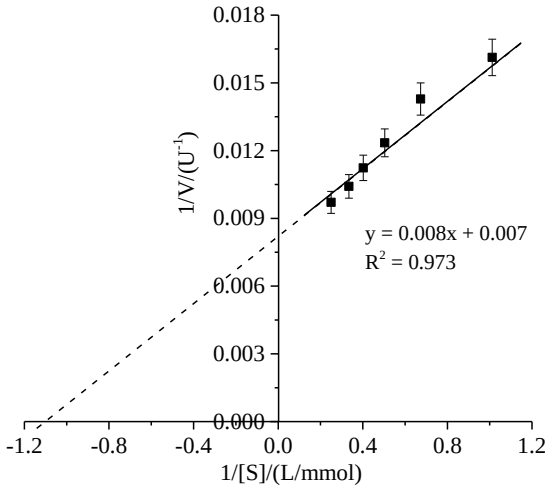

Fig. 5: Lineweaver-Burk plot for Km and Vmax determination of POD

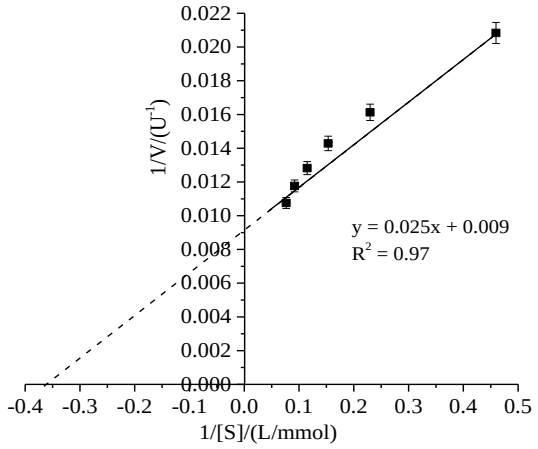

Fig. 6: Lineweaver-Burk plot for Km and Vmax determination of POD

Thermal Stability of POD

It is well known that peroxidase is one of the most heat-resistant enzymes compared with other enzymes (Serrano-Martínez *et al*., 2008). Peroxidase from different sources has different heat resistance (Damodaran and Parkin, 2017). The thermal stability curve of POD from leaves of *Brassica chinensis* L. treated at 100°C was shown in Fig. 7. It can be seen that the activity of POD decreased rapidly within a very short time (0-60 s) at 100°C. The activity of POD decreased steadily and approached inactivation for a period of time after that. The thermal deactivation curve consisted of a steep straight line part and a gentle straight line part (Fig. 7). From the shape of the thermal deactivation curve, it can be concluded that there were two independent first-order thermal deactivation reactions in the heat treatment process (Wu *et al*., 2023). The steep straight line represented the inactivation of the thermally unstable part of the POD. The gentle straight line represented the inactivation of the thermally stable part of the POD.

After heat treatment, the partial recovery of peroxidase activity during preservation at room temperature is enzyme regeneration, which is a characteristic of peroxidase. The temperature and time of heating and the temperature and time maintained after heating are the main factors that determine the regeneration of peroxidase activity (Adams *et al*., 2003). In this experiment, the method to solve the problem of enzyme regeneration was to cool the heat-treated peroxidase immediately in an ice bath to 0°C to ensure the reliability of the subsequent test data.

By extending the straight line to zero time of the heat resistant part of POD in Fig. 7, the proportion of the activity of the heat-resistant part of POD in the total enzyme activity can be estimated. In this experiment, the proportion of heat-unstable and heat resistant parts of POD was 98.91 and 1.09%, respectively.

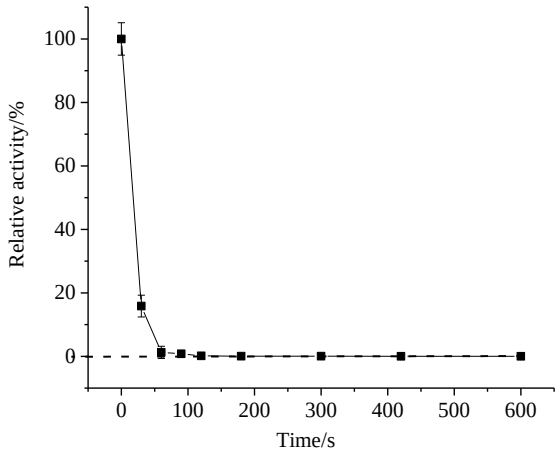

Fig. 7: Thermal stability of POD from leaves of *Brassica chinensis* L. treated at 100°C

Activation Energy of Thermal Denaturation of POD

The residual percentage activity of POD in leaves of *Brassica chinensis* L. under different blanching temperatures was shown in Fig. 8. The percentage residual activity of the samples treated at 20, 25, 30 and 35°C was basically unchanged, while the samples treated at 40 and 45°C changed slightly. When the temperature reached 50°C, with the extension of heat treatment time, the residual activity of POD changed obviously.

The kinetic curve of POD inactivation at different blanching temperatures is shown in Fig. 9. In the range of 20-45°C, the logarithm of residual activity of samples was basically unchanged and the logarithm of residual activity of samples treated at 50-60°C was slightly changed. When the temperature reached 70°C, the logarithm of residual activity of POD changed significantly with the extension of heat treatment time. The thermal deactivation rate constant k of the enzyme at each heat treatment temperature was obtained from Fig. 9, which was -0.01, -0.017, -0.026, -0.096, -0.312, -0.474, -0.446, -0.970, -1.85 and -4.38 min-1 respectively from 35°C. It can be seen that the thermal deactivation rate constant *k* of POD decreased rapidly after reaching the optimal reaction temperature of 40°C (-0.017). The k value changed rapidly between 70 and 80°C. The thermal deactivation rate constant k changed from -0.446 at 70 to -0.970 at 80°C, indicating that the denaturation temperature of the enzyme may occur between 70 and 80°C.

A straight line can be obtained by plotting the logarithm ln of the enzyme thermal inactivation rate constant k (absolute value) against 1/T (K's temperature). According to the Arrhenius equation k = Ae Ea/RT, the slope of the straight line was Ea/2.3R, where Ea was the activation energy of enzyme thermal denaturation (Wang *et al*., 2023). R is the universal gas constant (8.314 J/(moL*K)). The activation energy of POD thermal denaturation was 199.54 kJ/moL (Fig. 10).

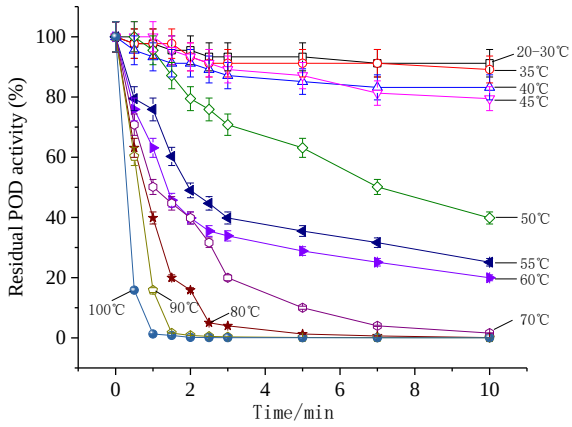

Fig. 8: Residual activity curves of POD at different blanching temperatures

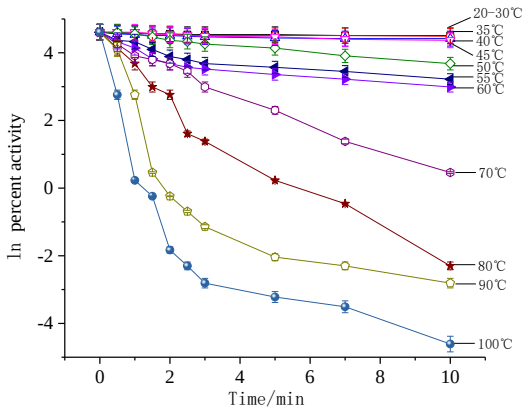

Fig. 9: The kinetic curve of POD inactivation at different blanching temperatures

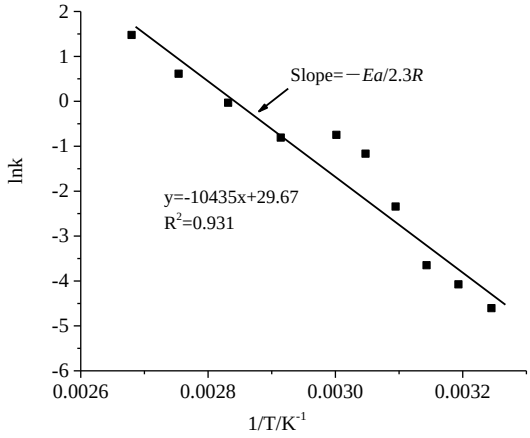

Fig. 10: Arrhenius plot of inactivation rates of POD

Similar Ea reports included lily bulb peroxidase (122.84 kJ/moL) (Wang *et al*., 2023), carrot peroxidase (151.40 kJ/moL) (Gonçalves *et al*., 2010), and red alga peroxidase (121.6 kJ/mol) (Fortea *et al*., 2011). It was worth noting that zucchini peroxidase had a high Ea of 925 kJ/moL (Neves *et al*., 2012).

Conclusion

Brassica chinensis L. has strong adaptability, high yield, and high quality and can be produced and supplied to the market annually with high economic benefits. It is a common vegetable variety in southern Jiangsu China with high yield and wide sales. With the rapid development of cross-border cold chain logistics technology, freshly picked *Brassica chinensis* L. can be transported through the cold chain and quickly sold to northern China and neighboring countries or regions such as Japan and Taiwan. In order to ensure product quality in cold chain management such as transportation, storage, and distribution, it is particularly important to know some enzymatic characteristics of POD, especially the effect of heat treatment on POD activity and its related kinetics.

The results showed that the POD activity was the highest at pH 6.8, which was very close to the pH during heat treatment. Compared with hot water blanching, the high-temperature short-term steam blanching of vegetables can shorten the time of heat treatment and related reactions, which is beneficial to the maintenance of vegetable quality (Drake and Carmichael, 1986).

The denaturation rate of POD from leaves of *Brassica chinensis* L. was different at different temperatures. The activation energy of POD was 199.54 kJ/moL and the activation energy Ea of the POD catalytic reaction was 35.34 kJ/mol. The optimum reaction temperature of POD was 40°C. The suitable growth temperature of *Brassica chinensis* L. was 15~22°C, while the environmental temperature during harvest in southern Jiangsu is generally 3~28°C. Therefore, the temperature of harvest has little influence on the quality of *Brassica chinensis* L. and the key lies in its postharvest heat treatment and logistics mode.

Similar to most known POD, the POD regeneration ability of from leaves of *Brassica chinensis* L. after heat treatment was strong. The ice bath cooling of the leaves after heat treatment can effectively reduce the regeneration of POD. Therefore, after blanching, the harvested fresh *Brassica chinensis* L. needs to be transported to the supermarket through the cold chain for sale.

Furthermore, the regeneration mechanism of POD from leaves of *Brassica chinensis* L. needs to be further studied. Understanding the regeneration mechanism of POD will help to optimize the heat treatment process parameters such as pressure, temperature, and time and clarify the temperature and time of low-temperature storage after heat treatment. The purpose of the present study is to provide a basis for cold chain logistics and quality control of *Brassica chinensis* L. It is expected that the relevant basic research data in this study can make a useful contribution to the quality control of *Brassica chinensis* L.

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Author's Contributions

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Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and that no ethical issues are involved.

Declarations

The authors declare that they have no competing interests.

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