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THERMODYNAMICS AND KINETICS OF THERMAL INACTIVATION OF PEROXIDASE FROM MANGOSTEEN (*GARCINIA MANGOSTANA* L.) PERICARP

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Abstract

Mangosteen (*Garcinia mangostana* L.) pericarp is an abundant source of phytochemicals. Blanching prior to further process stabilizes these valuable compounds. In this research, crude peroxidase (POD) was extracted from mangosteen peel using Triton X-100. Kinetics of POD inactivation was studied over temperature range of 60- 100°C. The inactivation kinetics followed a monophasic first-order model with k values between 1.93×10^{-2} - 8.14×10^{-2} min^{-1}. The decreasing trend of k values with increasing temperature indicates a faster inactivation of peroxidase from mangosteen pericarp at higher temperatures. The activation energy (E_a) of 35.06 kJ/mol was calculated from the slope of Arrhenius plot. Thermodynamic parameters (ΔH, ΔG, ΔS) for inactivation of peroxidase at different temperatures (60-100°C) were studied in detail. The results of this research will help to design pre-processing conditions of mangosteen pericarp as a source of antioxidants.

Keywords: Mangosteen, Peroxidase, Thermal inactivation, Kinetics, Thermodynamics.

1. Introduction

Mangosteen (*Garcinia mangostana* L.) is a tropical fruit, known as "the Queen of fruits", and consists of aril and pericarp. The aril, the edible part of fruit, is rich in vitamins, minerals, beta-carotene and beta- cryptoxanthin. Mangosteen pericarp,

Nomenclatures

C	Activity at time t
C_o	Initial activity at time zero
D	Decimal reduction time, min.
E_a	Activation energy, kJ/mol
h	Planck's constant
K_B	Boltzman constant
k	inactivation rate constant (min-1)
p	Probability
R	Universal gas constant, 8.314 J/mol. K
T	Absolute temperature, K
t	Time, min.
$t_{1/2}$	Half-life time, min

Greek Symbols

ΔG	Changes in Gibbs free energy, kJ/mol
ΔH	Changes in enthalpy, kJ/mol
ΔS	Changes in entropy, J/mol.K

composes 70% of fruit, is dark purple with a thickness of 6-10 mm [1, 2]. It is a good source of phenolic compounds, including xanthones [3], anthocyanins, proanthocyanins [1, 4], phenolic acids [1] and flavonoids [3-5].

The main phenolic compounds in the pericarp are xanthones [6]. Six major xanthones in pericarp are α-mangostin, β-mangostin, 9-hydroxycalabaxanthone, 3-isomangostin, gartanin, and 8-desoxygartanin [7]. Anthocyanins are responsible for the purple colour of pericarp, which are also important antioxidants. The major phenolic acid in mangosteen pericarp is protocatechuic acid [1]. Phenolic acids are not uniformly distributed in mangosteen fruit. The pericarp has more than 18 times higher total phenolic acid content than aril [1].

The beneficial effects of mangosteen pericarp such as anti-oxidant, anti-inflammatory and antibacterial activity are due to presence of natural antioxidants such as xanthones and anthocyanins [6, 8]. Besides, the high concentration of anthocyanins in the pericarp makes it a good choice as a natural food pigment [2]. The tea from mangosteen pericarp can help in digestion, fatigue and low energy. Due to high anthocyanin concentration in pericarp, it is a good choice to be used as a natural pigment [2].

However, enzymes such as peroxidase (POD) and polyphenol oxidase (PPO) can cause reduction in phenolic content of pericarp. For example enzymatic degradation caused higher losses of xanthones during drying at 60°C than hot-air drying at 75 and 90°C [7]. Any damages to mangosteen pericarp increase peroxide activity [9]. Thermal pretreatments such as blanching improve the retention of polyphenolics during processing and storage. POD, a haem-containing enzyme, occurs in most vegetables and fruits and is one of the most thermostable enzymes so generally it is used as the indicator to monitor blanching effectiveness [10].

Developing mathematical models of enzyme inactivation in heat-treated foods and calculation of its thermodynamic parameters provide the knowledge

on the enzyme thermal stability and enable us to determine the effect of different thermal treatments on remaining enzyme activity without the requirement of performing several trial runs [11]. To date, there is no data on the thermal behavior of POD in mangosteen pericarp. Therefore, in this research, we aimed at determining the kinetic and thermodynamic parameters for thermal inactivation of crude POD from mangosteen pericarp.

2. Materials and Methods

2.1. Materials

Fresh mangosteens (*Garcinia mangostana* L.), of commercial maturity, were purchased from a local market in Serdang, Malaysia. Fruits with dark purple peel were selected. All fruits were washed and the damaged ones were removed. Before each experiment, fruits were cut, with a sharp knife, in half and separated from the edible part.

2.2. Preparation of crude enzyme extract

Fresh mangosteen pericarps were homogenized with enzyme extract solution (1:6 v/w) in a blender for 2 min. The ratio between the extract solution volume (mL) and sample weight (g) was chosen based on preliminary experiments. The solution was a 0.1 M potassium phosphate buffer (pH 7) containing 4% (w/v) Polyvinylpolypyrrolidone (PVPP) and 1% (v/v) Triton X-100 [12]. PVPP is a not-specific phenolic absorbent and Triton X-100 was used to prevent the formation of tannin- protein complex. The homogenate was passed through cheesecloth and then filtered through Whatman No. 1 filter paper. The filtrate was centrifuged in a Kubota centrifuge, model 5800 (Kubota Corp., Tokyo, Japan), at 3300 rpm for 15 min at 4°C. The supernatant was collected as enzyme extract.

2.3. Thermal treatment of enzyme

The thermal inactivation of mangosteen peel crude POD was studied in the range of 60-100°C with different time of exposure between 2-12 min, which is normally sufficient for inactivate all enzymes present. To study heat inactivation, an aliquot of 5 mL enzyme extract was transferred to clean test tubes. Test tubes were put in a water bath of predetermined temperatures. To confirm the temperature of the water bath, a digital thermometer (Ellab CTD-85, Ellab, Denmark) was used. After preselected times, samples were cooled immediately in ice- water for 5 min. An aliquot of enzyme without thermal treatment was used as control.

2.4. Peroxidase activity assay

POD activity was determined, using a UV–Vis spectrophotometer (Genesys 10S UV–Vis Thermo Scientific, USA) based on the initial increase in absorbance at 470 nm. Modified method of Arnnok et al. [13] was used. Peroxidase substrate solution contained 0.15µl guaiacol, 0.15 µl hydrogen peroxide (30%), and 2.93 mL phosphate buffer (0.1 mol/L, pH 7). To measure peroxidase activity, 40 µl of enzyme extract was mixed with the substrate solution. The reaction was

monitored for 5 min at 5-s intervals. All experiments were performed in triplicate. Residual enzyme activity (RA) in heat-treated samples is expressed as a fraction of initial activity (C_0):

Residual enzyme activity = $C/C_0 \times 100$ (1)

where C and C_0 are ΔAbs./min after heat treatment at time t and time zero, respectively.

2.5. Calculation of kinetic parameters

The first-order equation, Eq. (2), was used to describe the enzyme inactivation in mangosteen pericarp [14].

$$\ln\frac{C}{C_0} = -kt \quad (2)$$

where C represents the value of enzyme activity at time t, C_0 is the initial value at time zero, k is the rate constant at the process temperature (min^{-1}), and t is time (min). The inactivation rate constant (k) was obtained from the slope of the semi-logarithmic plot of residual activity against the treatment time.

The half-life ($t_{1/2}$) was determined from following relationship, Eq. (3) [14]:

$$t_{1/2} = \frac{\ln(2)}{k} \quad (3)$$

Equation (4) directly relates D-value to the inactivation rate constant [14]:

$$D = \frac{ln(10)}{k} \quad (4)$$

The Z-value is the temperature needed to reduce the D-value one log-unit and it is obtained by plotting log D-value against corresponding temperature. The slope of the line is equal to negative reciprocal of Z-value.

Arrhenius Law, Eq. (3), describes the temperature dependence of the rate constant:

$$\ln(k) = \frac{E_a}{RT} + C \quad (5)$$

where E_a is the activation energy, R is the gas constant (8.314 J/mol.K), and T is the absolute temperature.

Using the values of the activation energy (E_a) and Arrhenius rate constant (k) different thermodynamic parameters were determined such as Gibbs free energy change (ΔG), the enthalpy change (ΔH) and the entropy change (ΔS) according to following expressions [15]:

$$\Delta H = \Delta E - RT \quad (6)$$

$$\Delta G = -RT\ln(kh/K_B\,T) \quad (7)$$

$$\Delta S = (\Delta H\text{-}\Delta G)/T \quad (8)$$

where K_B is Boltzmann's constant (1.3806×10^{-23} J/K), h is Planck's constant (6.6260×10^{-34} J.s)

2.6. Statistical analysis

An analysis of variance (two-way ANOVA with three replications) was performed to assess the blanching time–temperature conditions effect on peroxidase activity, with a value of $p < 0.05$ being considered statistically significant. All statistical analyses and linear regressions were performed using MINITAB (Version 14, Minitab Inc., PA, USA).

3. Results and Discussion

The rate of thermal inactivation of POD from mangosteen pericarp was measured over temperature range of 60-100°C. Treatment time and temperature had significant ($p < 0.05$) effect on the inactivation of POD, however, complete inactivation of POD could not be achieved. POD retained approximately 80 and 40% of its initial activity at temperatures 60-100°C, respectively, which indicates the presence of heat-resistant isoforms of peroxidase [16].

3.1. Thermal inactivation kinetics of peroxidase

The semi-log plots of the residual activity versus treatment time (Fig. 1) were linear at all temperatures, suggesting that the inactivation followed a simple first-order monophasic kinetic model (R^2 = 0.967-0.982).

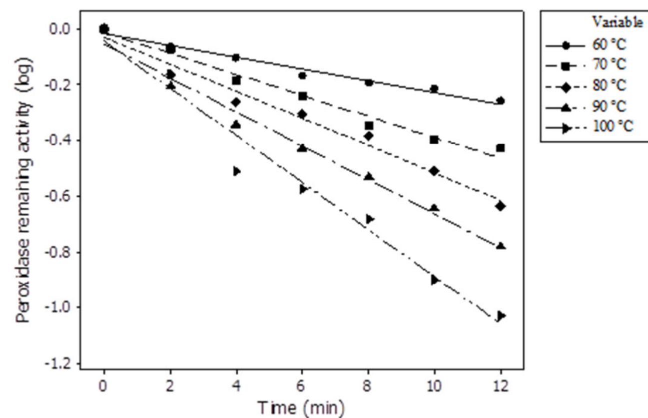

Fig. 1. Inactivation of Mangosteen Pericarp Peroxidase.

Table 1. *k*-, *D*-, $t_{1/2}$-Values for the Thermal Inactivation of Peroxidase in Mangosteen Pericarp in the 60-100 °C Temperature Range.

Temperature (°C)	*k* (min^{-1})	*D* (min)	$t_{1/2}$ (min)	R^2
60	0.0193 ± 0.0015	119.4	35.9	0.970
70	0.0353 ± 0.0012	65.2	19.6	0.975
80	0.0441 ± 0.0028	52.2	15.7	0.982
90	0.0583 ± 0.0025	39.5	11.9	0.979
100	0.0814 ± 0.0027	28.3	8.52	0.967

This result is in agreement with those obtained from carrot and potato [17], table grape [18], carrot [19] and seedless guava [10]. The presence of labile and resistant forms of POD is reported in a number of fruits and vegetables such as butternut squash [20] and pinto beans [21]. However, in this study only the resistant behavior was observed. It may be due to rapid inactivation of heat-labile fraction of the POD during the first seconds of heat treatment [20].

From the slope of the lines in Fig. 1, the inactivation rate constants (*k*) were calculated. The trend of changes in *k* values with increasing temperature indicates a faster inactivation of peroxidase at higher temperatures. The rate constant values increased with temperature from 1.93×10^{-2} to 8.14×10^{-2} min at 60-100°C, respectively. Estimated kinetic parameters for heat inactivation of POD from mangosteen pericarp are reported in Table 1.

D-value and half-life time exhibit the thermo-stability of the enzyme. The results showed that half-life and *D* value decreased with increase in temperature. Comparing the *D* and $t_{1/2}$ values of POD from other sources at the same temperature (80°C), mangosteen pericarp POD ($t_{1/2}$ = 15.7 min; *D* = 52.2 min) was more thermo stable than POD from strawberry ($t_{½}$ = 1.4 min) [22] and mature coconut water (*D* = 2.22 min) [23].

The dependence of the inactivation rate constants on temperature was fitted to Arrhenius Equation; Eq. (5), Fig. 2. This linearity suggests that the inactivation of POD of mangosteen pericarp happens via a unique temperature-dependent mechanism, such as protein unfolding [14]. The *Z*-value is the temperature increase needed for 90% of decrease in the *D*-value.

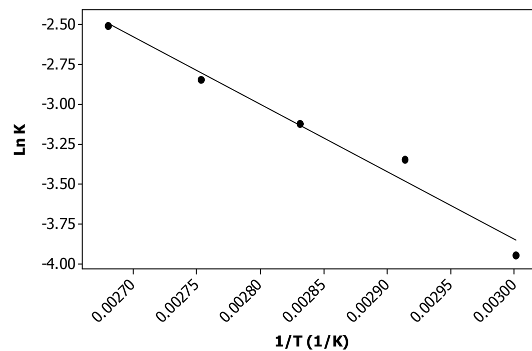

Fig. 2. Arrhenius Plot for Heat Inactivation of Mangosteen Pericarp Peroxidase.

Figure 3 presents the relationship between *D*-value and temperature. In general, higher *Z*-values reflect greater thermo stability of enzyme and lower activation energy (E_a) values indicate a greater stability of the enzyme to temperature change. E_a and *Z*-values for mangosteen pericarp POD were calculated as 35.06 ± 3.28 kJ/mol (R^2 = 0.974) and 68.03 ± 0.06°C (R^2 = 0.967), respectively. The estimated activation energy was quiet low compare to that reported for pumpkin (86.2 kJ/mol) [24], Elsanta strawberry (96.2 kJ/mol) [22] and melon (86.3 kJ/mol) [25]. However, PODs from *Rabdosia serra* leaf (20.15 kJ/mol) [26] and potato (27.11 kJ/mol) [27] showed lower E_a value. In the case of

Z-value, it was higher than reported value for POD from mature coconut water [23]. When it comes to compare the Z-values from different sources, it should be taken into consideration that Z-values might be influenced by the degree of ripeness as well as preparation method [28]. The E_a and Z-values from this study suggest that POD from mangosteen pericarp had quiet higher thermal stability compared to those from other sources.

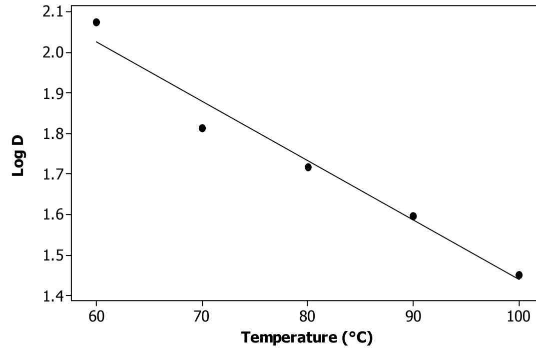

Fig. 3. Temperature Dependence of the Decimal Reduction Time for Inactivation of Mangosteen Pericarp Peroxidase.

3.2. Thermodynamic analysis of thermal denaturation

The information about thermodynamic parameters is a good tool to analyze the stability of protein and to reveal any secondary stabilization or destabilization effects that might be missed if only the half-life times are taken into consideration [14]. These parameters, namely Gibbs free energy change (ΔG), the enthalpy change (ΔH) and the entropy change (ΔS), are presented in Table 2.

Table 2. Thermodynamic Parameters for the Thermal Inactivation of Crude Peroxidase in Mangosteen Pericarp.

Temperature (°C)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol.K)
60	32.290	104.17	-215.77
70	32.207	105.66	-214.06
80	32.124	108.17	-215.34
90	32.040	110.48	-215.99
100	31.957	112.57	-216.03
Mean	32.124±0.131	108.21±3.42	-215.44±0.82

In this study, ΔG was in the range of 104.173 to 112.568 kJ/mol at 60-100°C. The values were very similar at about 100 kJ/mol, which is the characteristic of protein denaturation reaction [14]. ΔG is the energy barrier for enzyme inactivation and is a measure of the spontaneity of the inactivation process [15].

In other words, the higher the ΔG is, the more stable is the enzyme [14]. It can be said that POD from different variety of strawberry with ΔG of about 370 kJ/mol [22] is more stable than POD from mangosteen pericarp.

The average of ΔS and ΔH values for POD thermo-denaturation process was -215.44 J/mol.K and 32.12 kJ/mol, respectively. When the enzyme molecule folds/unfolds from its stable native structure, the randomness of the system, entropy, changes [29]. The entropy change (ΔS) indicates the net enzyme and solvent disorder [14,15]. The possible reason for negative ΔS of our data is an increase in order of the system through an aggregation process in which a few inter- and/intramolecular bonds are formed [30]. The negative entropies changes during inactivation are in accord with the compaction of the enzyme molecule [29]. ΔH, the enthalpy change, is a measure of the number of bonds broken during inactivation [14,15]. The positive values of ΔH indicate that denaturation of mangosteen peel peroxidase is an endothermic reaction. Positive values of ΔH have also been reported for peroxidase from strawberry [22] and kinnow [31]. The two main factors that affect the numerical values of ΔS and ΔH are solvent and structural effects [29]. It is worth mentioning that isolated values for ΔS or ΔH are not good predictors of enzyme stability [15].

4. Conclusions

Thermal stability of crude peroxidase from mangosteen (*Garcinia mangostana* L.) pericarp was studied over a temperature range of 60- 100°C. Thermal treatments leaded to partial inactivation of the enzyme. The major conclusions of this study are given below.

- Mono-phasic first order model well described enzyme inactivation.
- Considering the obtained kinetics and thermodynamics values, mangosteen pericarp POD belongs to the group of heat stable enzymes. The relatively high thermostability of POD could be taken into account when thermal treatments are applied to obtain processed products derived from mangosteen (*Garcinia mangostana* L.) pericarp.

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