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KINETICS OF CRUDE PEROXIDASE INACTIVATION FROM MANGOSTEEN een (*Garcinia mangostana* L.) PERICARP BY USING THERMAL TREATMENT

Mahsa Ziabakhsh Deylami[1], Russly Abdul Rahman[1,2,3] , Jamilah Bakar[1], Lasekan Olusegun[1], Tan Chin Ping[1]

[1] Department of Food Technology, Faculty of Food Science and Technology, University Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia.
[2] Halal Product Research Institute, University Putra Malaysia, 43400 UPM Serdang, Selangor D. E., Malaysia.
[3]Department of Process and Food Engineering, Faculty of Engineering, University Putra Malaysia, 43400 Serdang, Selangor D.E., Malaysia.
Email: Mahsazd@yahoo.com

ABSTRACT

Mangosteen (*Garcinia mangostana* L.) pericarp is a good source of phytochemicals that could be used as a valuable food ingredient. Blanching prior to further process can help to preserve phytochemicals. In this research, kinetics of crud peroxidase (POD) inactivation from mangosteen pericarp was investigated. Hot water blanching at temperature range of 70-100°C for 11min was applied. Kinetics of enzyme inactivation followed monophasic first-order model (R^2=0.977-0.985) suggesting rapid inactivation of heat-labile fraction of the enzyme during the first seconds of treatment. The inactivation rate constant was 0.1189min^{-1} and 0.2424min^{-1} for 85℃ and 100℃, respectively. The activation energy (E_a) forperoxidase inactivation was 6699.9$Jmol^{-1}$.The result of this work is a good tool to optimize thermal treatment of mangosteen pericarp.

Keywords*: Peroxidase, Thermal inactivation, Mangosteen Pericarp, Kinetics parameters, Blanching.*

INTRODUCTION

Mangosteen (*Garcinia mangostana* L.) is a tropical fruit belonging to Guttiferae family. Aril, the edible portion of fruit, is white, soft and sweet which is a source of minerals, vitamins, beta-carotene and beta-cryptoxanthin. Mangosteen pericarp is dark purple, 6-10mm in thickness and composes 70% of fruit [1,2]. The pericarp is a rich source of phenolic compounds, including xanthons, anthocyanins, proanthocyanins, phenolic acids and flavonoids [3,4,5]. Besides, it is a good source of pectin

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Xanthons are main phenolic compounds in pericarp .They have anti-oxidant, anti-inflammatory, antibacterial, anti-malarial and anti-fungal activities [6,7].Six xanthones are present in pericarp, which are α-mangostin, β-mangostin, 9-hydroxycalabaxanthone, 3-isomangostin, gartanin, and 8-desoxygartanin[8].Anthocyanins, responsible for purple colour of pericarp, have been reported to have antioxidant activity. Cyanidin-3-sophoroside and cyaniding-3-glucoside compose major anthocyanins in the pericarp and their amount in pericarp increase with fruit colour development [9].Phenolic acids in mangosteen fruit are mainly present in pericarp [1]. Total phenolic acid content in pericarp is more than 18 times higher than that reported for aril [1].Protocatechuic acid is the major phenolic acid in mangosteen pericarp[1].

The pericarp can potentially be used as a therapeutic agent or as a functional food additive. In traditional medicine, the pericarp has been used to treat diarrhea, dysenteries [5]. 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 and polyphenoloxidase can cause decrease in phenolic content of pericarp. According to the findings of Suvarankuta *et al.*[8] enzymatic degradation led to higher losses of xanthones during drying at 60°C than hot-air drying at 75 and 90°C.Pretreatments such as blanching can inhibit or minimize the degradation of polyphenolics. Since POD is one of the most heat-stable enzyme and can be find

in high concentration in most vegetables and fruits, it is generally considered as the indicator of blanching effectiveness [10].

Mathematical modeling of enzyme inactivation in heated foods provides the opportunity to assess the effect of different heat treatments on residual enzyme activity without performing several preliminary runs [11]. There is no data on the kinetics of thermal inactivation of peroxidase in mangosteen pericarp. The aim of this study was to determine the kinetic parameters for crude peroxidase inactivation of mangosteen pericarp.

MATERIALS AND METHODS

Materials

Fresh mangosteens (*Garcinia mangostana* L.) were purchased from a local market in Serdang, Malysia. Fruits with complete maturation state were selected through peel colour (dark purple). All fruits were cleaned and the damaged ones were removed. Before each experiment, the peel was cut manually in half, separated from the aril. Table 1 presents the initial characteristics of various mangosteen fruit parts.

pH, Soluble Solids, Titratable Acidity and Moisture Content

The aril juice was used to measure pH, soluble solids content (SSC) and titratable acidity (TA). To extract the juice, the white flesh with the enclosed seeds was wrapped in cheesecloth and squeezed by hand. The pH (pH Meter Mettler-S20 SevenEasy, USA) was measured at 20°C. Soluble solids content was determined at 20°C using a refractometer (Atago-Master-20 M, Japan) and reported as °Brix. TA was determined from a 5mL aliquot by titration with 0.1M NaOH, using phenolphthalein as an indicator. Results are expressed as grams of citric acid per 100 mL.
To measure the pH of pericarp, 15 g of pericarppast was blended with 45 ml of distilled water for 2 min and then filteredthrough Whatman No. 1 filter paper (Whatman, Maidstone, UK). The pH of filtrate (pH Meter Mettler-S20 Seven Easy, USA) was measured at 20°C. Moisture content was determined by heating in a drying oven at 105°C for 48 h [12].All assays were performed in triplicate.

Thermal Treatment

Mangosteen pericarps were blanched in a thermostatic water bath (Memmert, WNE14. Memmert GmbH Co. KG, Germany) (± 0.5°C) in the range of 70-100°C, with different times of exposure between 1 and 11 min. After predetermined times, the samples were removed from the water bath and immersed immediately in iced water for 5min. During the heat treatments, the temperatures of the water bath were monitored by means of a digital thermometer (Ellab CTD-85, Ellab, Denmark). An unblanched sample was taken as control.

Extraction and Determination of Crude Peroxidase Activity

To extract POD, five grams of mangosteen pericarp paste were extracted with 0.1 M phosphate buffer pH 7. The homogenate was filtered through Whatman No. 1 filter paper. The filtrate was centrifuged in a Beckman Coulter, Avanti J-25 centrifuge with a rotor no.JA14 at 2,500 rpm and 4°C for 20 min with polypropylene tubes. The supernatants were collected as enzyme extract [13].
PPO activity was determined, using an UV/vis spectrophotometer (UV-mini 1240, Shimadzu Corporation, Japan), from the initial increase in absorbance at 470 nm. Peroxidase substrate solution contained 0.1 mL guaiacol, 0.1 mL hydrogen peroxide (30%), and 99.8 mL phosphate buffer (0.1 mol/L, pH7). To measure peroxidase activity, 3.48 mL of the substrate solution was mixed with 0.12 mL of enzyme extract in a 10-mm path length quartz cuvette [10]. The blank sample contained the same mixture solution without the enzyme extract. The reaction was monitored for 15 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):

$$\text{Residual enzyme activity} = C/C_0 \times 100 \quad (1)$$

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

Calculation of Kinetic Parameters

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

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

Where C represents the value of peroxidase activity at time t,C_0 is the initial value at time zero, k is the rate constant at the process temperature, and t is time. The inactivation rate constant (k) was obtained from the slope of the semi-logarithmic plot of residual activity against the treatment time. An Arrhenius Law (Eq. 3) describes the temperature dependence of the rate constant:

$$\ln(k) = \frac{E_a}{RT} + C \quad (3)$$

Where E_a is the activation energy, R is the gas constant (8.314 $Jmol^{-1}$ K^{-1}), and T is the temperature in K.

Statistical Analysis

An analysis of variance (two-way ANOVA with replication) 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 were performed using MINITAB (Version 14, Minitab Inc., PA, USA). The regression data were calculated by using Data Analysis in Microsoft Excel 2011.

RESULTS AND DISCUSSIONS

Mangosteen pericarps were treated using hot water in the temperature range of 70-100°C. 'Fig.1' shows residual activities as a function of time at three different temperatures. Enzymatic activity decreased with increasing temperature and treatment time. Temperature and treatment time had significant ($P < 0.05$) effect on the inactivation of mangosteen pericarp POD (Table 2).Similar results were reported for peroxidase from different sources by Gonçalves *et al.* [14,15] and Ganjloo *et al*.[10].

Complete inactivation of peroxidase often leads to over blanching [10]. 90% reduction in the peroxidase activity is recommended for optimum quality retention of fruits and vegetables. Residual peroxidase activities after 9min and 12min of blanching at 100°C were 12.695% and 7.277%, respectively.

Table1: Initial characteristics of different parts of mangosteen

Fruit part	Properties	
Pericarp	Diameter [mm]	48.67 ± 3.67
	Thickness [mm]	7.94 ± 1.07
	Moisture Content [%]	40.83 ± 0.55
	pH	4.09 ± 0.02
Aril	Total Soluble Solids [□Brix]	16.4 ± 0.01
	Titratable Acidity [%]	0.53 ± 0.02
	pH	3.41 ± 0.02

Table 2: Analysis of variance for peroxidase inactivation inmangosteen pericarp by thermal treatment

Source	DF	SS	MS	F	P
Time	6	6.1117	1.01862	6.37	0.003
Temperature	2	3.4193	1.70964	10.69	0.002
Error	12	1.9199	0.15999		

The semi logarithmic relationship between the residual activity of POD and treatment time was linear for all temperatures. Experimental results obtained in this work were well described by an Arrhenius first-order kinetic model (R^2=0.977-0.985).Presence of labile and resistant forms of POD is reported in a number of fruits and vegetables such as butternut squash [16] and pinto beans [17]. However, in this work only the resistant

behavior was observed. It is due to rapid inactivation of heat-labile fraction of the enzyme during the first seconds of treatment [16].This result is in agreement with those obtained from carrot or potato [18], table grape [19], carrot [14] and seedless guava [10].

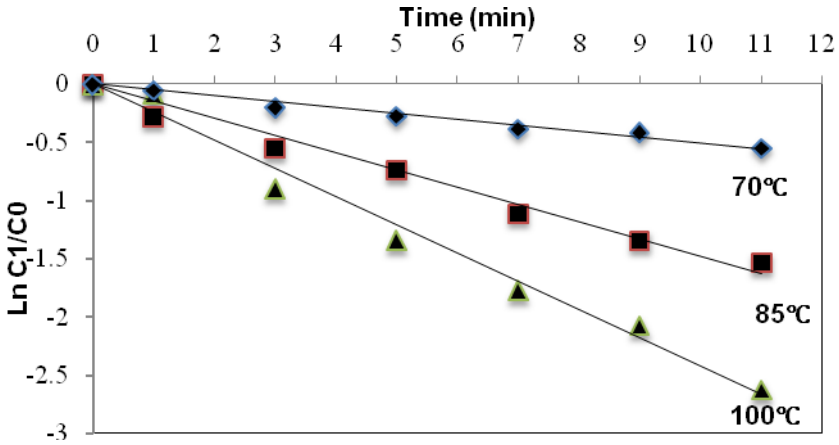

Fig.1: thermal inactivation of seedless guava peroxidase. Remaining peroxidase activity versus heating time

From the slopes of these lines, the inactivation rate constants (*k*) were calculated. The *k* values increased with temperature from 5.05×10^{-2} at 70°C to0.2424 min^{-1} at100°C.Estimated kinetic parameters are reported in Table2. The obtained activation energy of peroxidase inactivation was 6699.9 J mol^{-1} (R^2=9991).POD kinetic values have shown a wide variation, depending on the vegetable, initial concentration of enzyme and its distribution on product as well as presence of different isoenzymes[16]. Comparing the inactivation data obtained in this work with those of peroxidase from different sources, mangosteen pericarp peroxidase appears to be less resistant compared to peroxidase from carrot or potato [18], carrot [14], pumpkin [20] and seed less guava [10].

Table3: Kinetic parameters of mangosteen pericarp peroxidase inactivation due to blanching

Temperature [°C]	*K* [s^{-1}]	R^2	E_a[$Jmol^{-1}$]
70	0.0505±0.0012	0.9776	
85	0.1189±0.0040	0.9780	
100	0.2424±0.0092	0.9849	
			6699.9±6.2

CONCLUSIONS

Inactivation of peroxidase in blanched mangosteen pericarp followed monophasic first-order model. The Arrhenius model described the temperature dependence of the reaction rate constant. With the estimated kinetic parameters, the prediction of the residual POD activity for a given set of time–temperature conditions would be possible.

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