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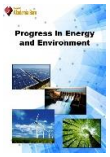

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Original Article

Evaluation of Thermal Degradation Kinetic Order of Anthocyanins Extracted from *Garcinia Mangostana* L. rind

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Abstract

Garcinia Mangostana L. rind is well-known for its natural occurrence anthocyanins red pigment. It has been extensively sought nowadays due to its abundant health benefited values. This study aimed to evaluate thermal degradation kinetic of anthocyanins extracted from *Garcinia Mangostana* L. rind at elevated storage temperatures. Total monomeric anthocyanins (TMA) of *Garcinia Mangostana* L. rind extract was quantified using pH differential method. Graphical integrated rate laws of zero-, first-, and second-order were used to evaluate the best fit of experimental data. Temperature dependency of anthocyanins was further investigated using natural logarithm Arrhenius plots. Results showed that TMA values were degraded significantly by 14.84%, 19.44%, 23.97%, and 29.61% when storing at temperatures of 25°C, 40°C, 55°C and 70°C, respectively, but merely 7.04% degraded at 10°C. This result indicated that 10°C storage condition is ideal for long term storage whereas 25°C storage condition is suitable for short term. Arrhenius plot with the highest R^2 value of 0.9761 has identified that second-order was the best model to describe anthocyanin thermal degradation from 10°C to 70°C. Activation energy (Ea) of anthocyanin thermal degradation was 22.54kJ/mol and half-lives were from 44.16 to 8.91 hour in temperature range from 10 to 70°C.

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1 Introduction

Anthocyanins are great source to the pharmacological field. It has been proven the ability to stop the stomach related catalysts, hindering the insulin by separating the pancreatic assault and is able to instigate the harms on DNA caused by malignancy cells [1]. Additionally, high anthocyanin content originating from natural plant materials gave great anti-inflammatory impacts [2]. For these reasons, anthocyanins have been extensively sought recently. The sought is no longer limited to edible part of pulp, but has been explored to flower petal [3,4], fruit rind [5,6] and even pomace [7]. Many studies have attempted anthocyanins incorporation to food [8] and beverage [9] in order to have a better reach to consumer of their potential health benefits. Anthocyanins extracted from Sohiong (*Prunus nepalensis* L.) [8] and purple carrot [9] have been attempted to incorporate into to yoghurt [8], syrup [8], hard boiled candy [8], and vitamin C rich commercial beverage [9]. Nevertheless, anthocyanins are highly susceptible to degradation by environmental conditions, such as temperature [4,6,10-13], light [6], pH [14], solid concentrate [15], water activity [16], and preservative [17]. Hence, many studies have

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emerged recently to tackle these problems, especially temperature effect. Thermal degradation kinetic behaviour has been widely studied as this information is vital to food processing industries for prediction of food shelf-life in order to ensure food security [4,6,10-13]. Selection of optimum processing temperature could preserve health benefited value in food. For instance, Martynenko and Chen [13] recommended the use of hydothermodynamic (HTD) processing to mitigate the effect of temperature on anthocyanins of blueberry puree. They revealed that HTD processed blueberry puree had longer shelf-life of 1.5 years for anthocyanin retention.

Among the plant materials, *Garcinia Mangostana* L. (mangosteen) rind has been discovered rich in anthocyanins [5] [6], besides its well-known antitumoral properties of xanthones [18]. However, the rind is normally disposed of as waste due to its unpleasant taste of bitterness. Knowing the health benefited values it contains, this work aimed to recover the anthocyanins from *Garcinia Mangostana* L. rind and determine the anthocyanins degradation kinetic order at elevated storage temperatures. Graphical integrated rate law and natural logarithm Arrhenius equations were used to evaluate the degradation kinetic order. Subsequently, activation energy, half-life and temperature quotient were calculated.

2 Materials and Method

2.1 Preparation of Garcinia mangostana l. rind powder

Garcinia Mangostana L. rinds were collected from a private orchard located in Negeri Sembilan, Malaysia. The rind was further processed by removing the ruptured part of the rind. Only the fresh rind was used in the experiment. 100 g of rind was weighed on an electronic weighing balance. Then the rind was dried under the fan at room temperature for 2 days. The dried rind was grounded into powder form (roughly 1mm), packed into a plastic bag and kept inside the refrigerator at 4 ± 0.5°C until the experiment.

2.2 Extraction of anthocyanin

The extraction of anthocyanins from *Garcinia mangostana* L. rind was carried out following method described by Cheok et al. [5] with a slight modification. In brief, 1 g of *Garcinia mangostana* L. rind powder was subjected into 100 mL of 50% ethanolic aqueous solution acidified with 2% citric acid (ethanol/water/citric acid, 50:48:2, v/v). The extraction was conducted in two conditions, i.e., stirring and without stirring, on a magnetic stirring plate. After one-hour duration of extraction, the mixture was filtered using Whatman filter paper and the extract was collected for total monomeric anthocyanin (TMA) determination. The experiment was conducted in duplicate.

2.3 Total monomeric anthocyanin determination

Total monomeric anthocyanin (TMA) was determined using pH-differential method. The extract was diluted to a dilution factor (DF) of 5 with buffer solutions. 2 mL of extract was mixed with 8 mL of buffer solution which is composed of 0.025M potassium chloride at pH1.0. Another 2mL of extract was mixed too but with 0.4M sodium acetate at pH4.5. The absorbance value for the diluted extracts were measured using UV-VIS spectrophotometer at 510 nm and 700 nm against distilled water as blank. The total monomeric anthocyanins can be calculated as cyanidin-3-glucoside using equation Eq. (1) [5,17].

$$\text{TMA(mg/L)} = \frac{A \times MW \times DF \times 1000}{e \times 1} \quad (1)$$

where A, absorbance = $(A_{510} - A_{700})_{pH1.0} - (A_{510} - A_{700})_{pH4.5}$, MW, molecular weight = 449.2 g/mole, DF = dilution factor, e = molar absorptivity coefficient is 26900 L/mol/cm, 1= path length in cm. The total monomeric anthocyanin (TMA) was further converged to milligram of cyanidin-3-glucoside per gram of rind powder (mg cy-3-glu/g) by dividing with solid to solvent ratio of 0.01g/ml.

2.4 Anthocyanin degradation kinetics order evaluation studies

Temperature dependency of anthocyanin extract was carried out at five different storage temperatures of 10°C, 25°C, 40°C, 55°C and 70°C. Extract was obtained by subjecting 1 g of mangosteen rind powder into 100 mL of 50% ethanolic aqueous solution acidified with 2% citric acid and stirred on a magnetic stirring plate for an hour. After filtration, a beaker contains 50 mL of anthocyanin extract (TMA_0 = 1.48 ± 0.05 mg/g) was covered with aluminium foil and stored at each storage temperature for a duration of 4 hours. Extract was determined its TMA value prior subjecting to storage at varying temperature conditions. One millilitre of extract was taken out at every hour interval for TMA determination and the measurement was done in triplicate. In order to determine the order of anthocyanins degradation, data obtained were plotted according to integrated rate laws [19], at each storage temperature:

$$\text{Zero-order: } (\text{TMA})_t = -kt + (A)_0 \tag{2}$$

$$\text{First-order: } \ln(\text{TMA})_t = -kt + \ln(A)_0 \tag{3}$$

$$\text{Second-order: } 1/\ln(\text{TMA})_t = -kt + 1/(A)_0 \tag{4}$$

where $(TMA)_t$ = anthocyanins concentration (mg cy-3-glu/g) at time t; k = reaction rate constant (h^{-1}); $(A)_0$, $\ln(A)_0$, and $1/(A)_0$ were intercepts of zero-, first- and second-order. Subsequently, half-life was calculated using either equation Eq. (5), (6), or (7) [19], according to the order of reaction determined based on higher R^2 value.

$$\text{Zero-order: } t_{1/2} = (TMA)_0 / 2k \tag{5}$$

$$\text{First-order: } t_{1/2} = -\ln 0.5 / k \tag{6}$$

$$\text{Second-order: } t_{1/2} = 1/k(TMA)_0 \tag{7}$$

where k = reaction rate constant (h^{-1}); $t_{1/2}$= half−life (h); $(TMA)_0$ = initial anthocyanins concentration (mg cy-3-glu/g).

Mangosteen extract was further investigated for activation energy using graphical method of natural logarithm Arrhenius equation (Eq. 8) [19] and temperature quotient value (Q_{10}) [6,10,13,17] was calculated using Eq. (9) to characterize the effect of of temperature on the rate of anthocyanin degradation.

$$\ln k = \frac{-E_a}{R}\left(\frac{1}{T}\right) + \ln A \tag{8}$$

$$Q_{10} = \left(\frac{k_2}{k_1}\right)^{10/(T_2 - T_1)} \tag{9}$$

where E_a= activation energy (J/mol); R = 8.314 (J/mol.K); k = reaction rate constant (h^{-1}); A = frequency factor; T = Temperature (K); Q_{10} = the temperature coefficient (K^{-1}); k_1 and k_2 = rate constant (h^{-1}) at temperature T_1 and T_2 (K).

2.5 Statistical analysis

Data were analysed using Microsoft Excel Data Analysis's single factor ANOVA (Analysis of Variance) for significant difference by setting 95% confidence level ($p < 0.05$). Result is expressed as mean value ± standard deviation (n = 8). Coefficient of determination (R^2) was performed to evaluate linear regression of experimental data good fit.

3 Results and Discussion

3.1 TMA Yields from extraction conditions

Fig. 1 shows that TMA yields of *Garcinia Mangostana* L. extracted under stirring condition (1.18 ± 0.0758 mg cy-3-glu/g) was 62.83% significantly higher ($p < 0.05$) than without stirring (0.439 ± 0.0453 mg cy-3-gly/g). This can be explained as extraction happens under diffusion-controlled condition, stirring of the sample mixture was able to accelerate the extraction by favoring diffusion of the analytes from the bulk solution to the extractant phase [20]. In addition, this phenomenon can be explained by Nernst law where the diffusion through the boundary layer between the *Garcinia Mangostana* L. rind powder and the extraction solution is rate controlling which can be enhanced by efficient stirring of the solution [20]. Hence, this study has proven the efficiency of the stirring condition on anthocyanin extraction from *Garcinia Mangostana* L. rind powder.

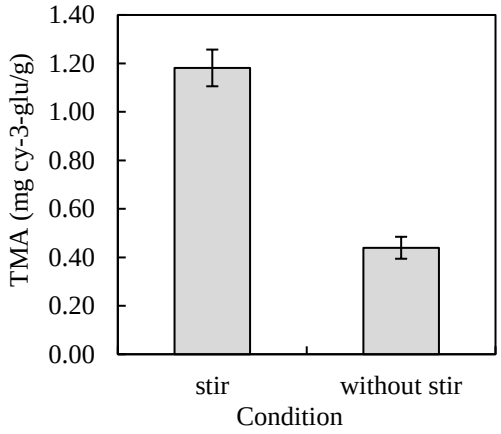

Fig. 1 Total monomeric anthocyanin (TMA) yields obtained from *Garcinia Mangostana* L. rind under different extraction conditions.

3.2 Effect of temperature on TMA degradation

Result clearly showed that no significance ($p > 0.05$) degradation of TMA value extracted from *Garcinia Mangostana* L. rind under the storage at 10 °C for the whole duration of 4 hours (Fig. 1a). However, a significance degradation ($p < 0.05$) of TMA was observed for extracts stored at 25°C (Fig. 2b) and 40°C (Fig. 2c), respectively, at the 4th hour. At storage temperatures of 55°C and 70°C, TMA values of *Garcinia Mangostana* L. rind extract started to degrade significantly ($p < 0.05$) at the 3rd and 2nd hour, respectively. Result indicated that the higher temperature, the shorter the storage time for the extract. Besides, higher storage temperature promotes higher degradation percentage of TMA. Degradation percentage was calculated from the difference of initial TMA concentration (TMA_o) and at the hour where TMA value started degraded significantly (TMA_t) divided by the initial TMA concentration:

$$\% \text{ degradation } = \frac{(TMA)_0 - (TMA)_t}{(TMA)_0} \tag{10}$$

TMA was observed degraded by 14.84%, 19.44%, 23.97%, and 29.61% at 25°C, 40°C, 55°C and 70°C, respectively, while merely 7.04% degraded at 10°C which was no statistically significance. Hence, *Garcinia Mangostana* L. rind extract is recommended to store at 10°C for a better preservation of TMA value. This result also indicated that 10 °C storage condition is ideal for long term storage whereas 25°C storage condition is viable for short term storage.

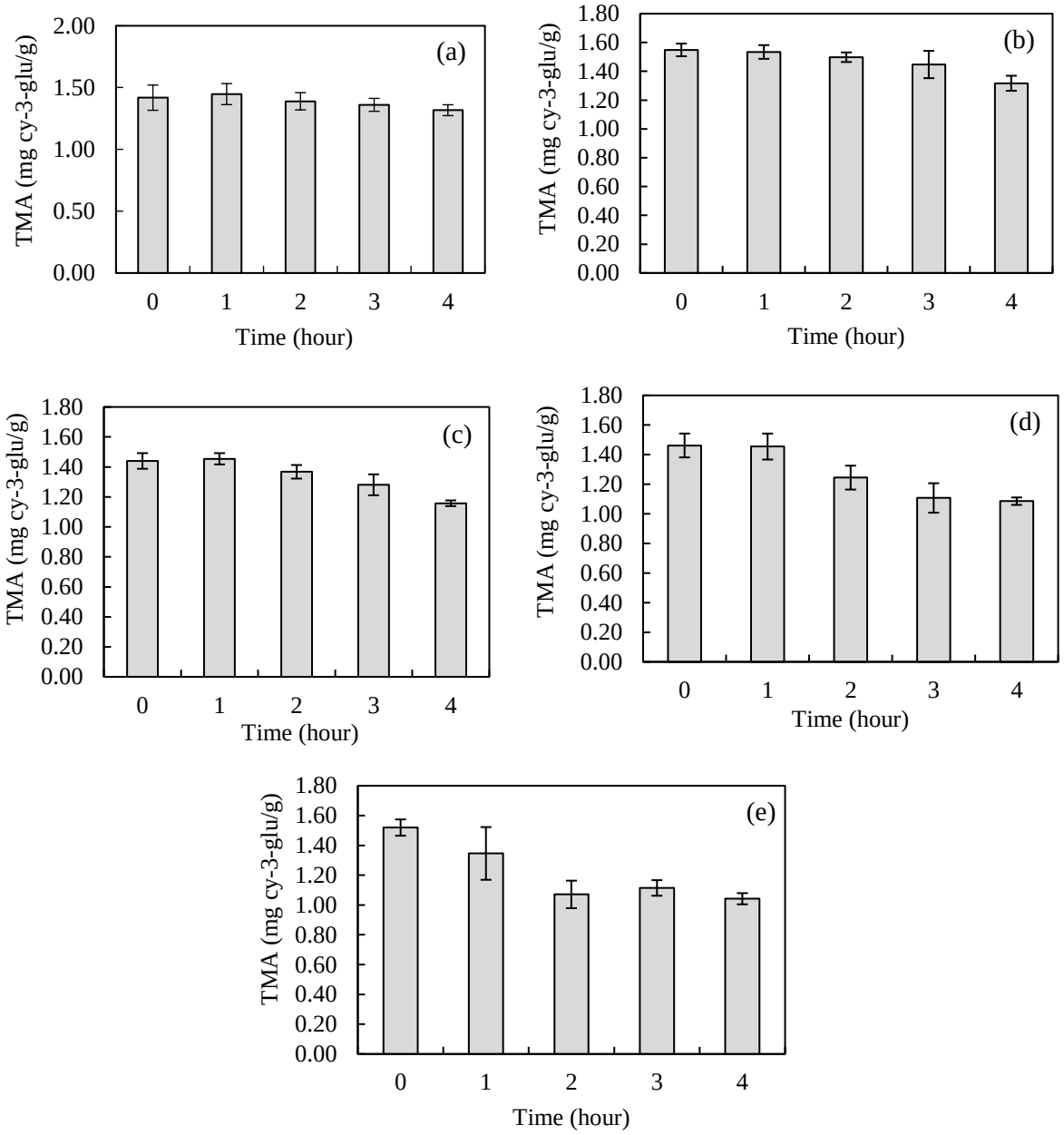

Fig. 2 Effect of storage temperatures of (a) 10°C, (b) 25°C, (c) 40°C, (d) 55°C and (e) 70°C on TMA values of *Garcinia Mangostana* L. rind extract for 4 hours duration.

This result is in agreement with a previous study done by Chisté et al. [6]. They observed that long-term storage of the anthocyanin extracts from mangosteen peel at temperatures of 40 °C and 50 °C promoted faster degradation than the storage at room temperature of 28 °C. They discovered that under chill or frozen storage, the anthocyanin extracts showed high stability.

3.3 Thermal degradation kinetics order of anthocyanins

Fig. 3 shows plots of anthocyanins degradation of zero-, first-, and second-order at storage temperatures of 10°C, 25°C, 40°C, 55°C, and 70°C, using integrated rate laws. Result showed that coefficient determinations (R^2) obtained at 10°C was lower than 25°C, 40°C, 55°C, and 70°C. This is most probably because the extract showed no significance decrease of TMA value at storage of 10°C for the

4 hours duration. No significance R^2 values were observed from the integrated rate law plots. Hence, thermal degradation of anthocyanins kinetic order cannot be determined from these plots.

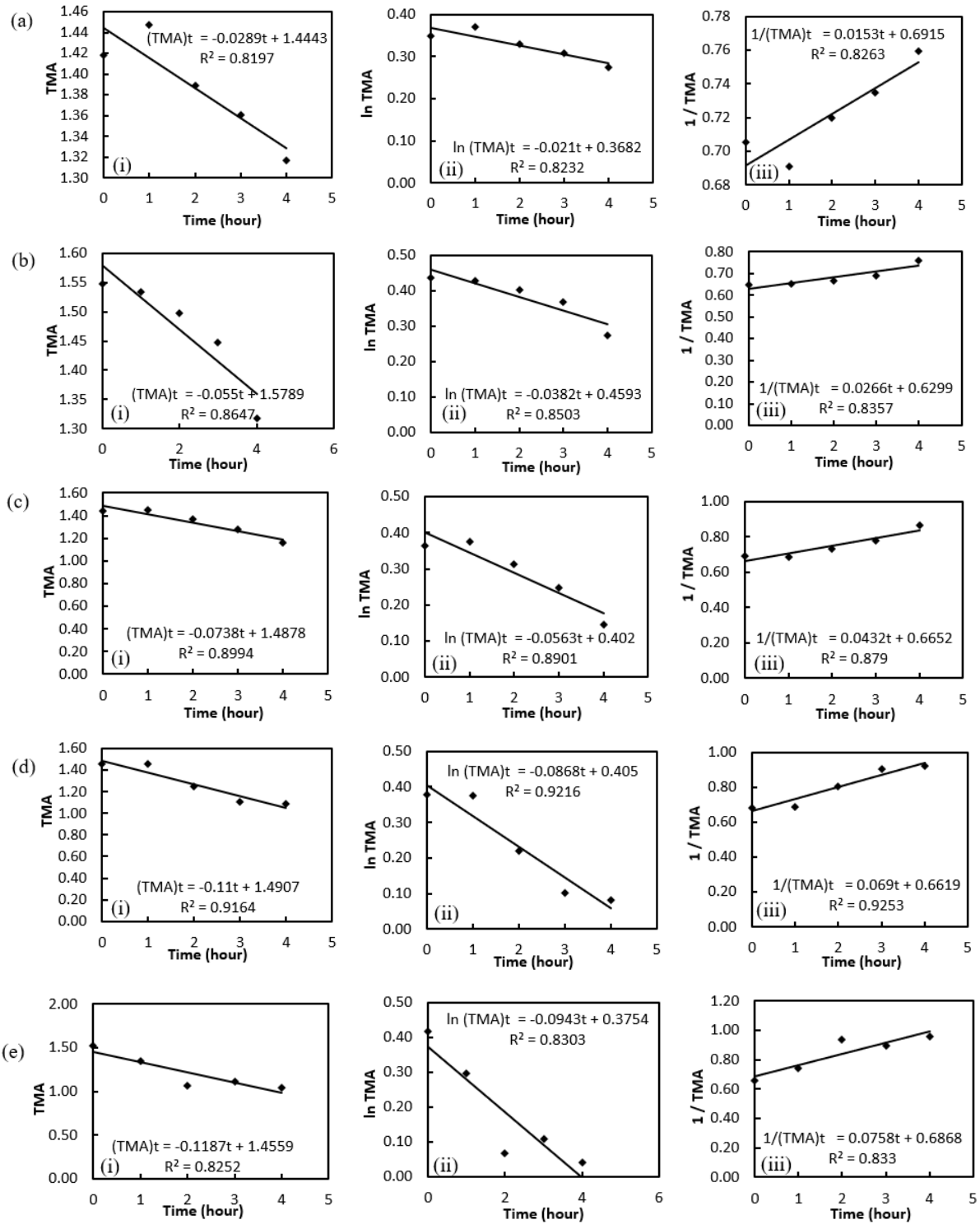

Fig. 3 Plots of zero (i), first (ii) and second (iii) degradation order of anthocyanins extracted from *Garcinia Mangostana* L. rind under temperatures of (a) 10°C, (b) 25°C, (c) 40°C, (d) 55°C and (e) 70°C.

Rate constant (k) increases with increasing temperature, which shows its strong dependency to temperature (Fig. 3). Therefore, natural logarithm Arrhenius plots (Fig. 4) were constructed using the

k values (slopes) obtained from zero-, first-, and second-order at each temperature level (Fig. 3). Fig. 4 showed that second-order model has the highest value of determination of coefficient (R^2 = 0.9761), in comparison to zero- (R^2 = 0.9578) and first-order (R^2 = 0.9701). This indicated that thermal degradation of anthocyanins extracted from *Garcinia Mangostana* L. rind followed second-order kinetic. Up to date, no studies have reported the use of natural logarithm Arrhenius plots to determine kinetic order. The best fit of experimental data for a kinetic model was determined based on higher R^2 values [6,12]. A study has been conducted by Chisté et al. [6] to find the best fit models (zero and first order) for the degradation of anthocyanins in mangosteen peel under different illumination conditions. They reported that first-order reaction model represented the best experimental data fit for all the illumination sources based on higher R^2 values and p values smaller than 5. First order and Weibull models have been tested for describing thermal degradation of anthocyanins extracted from mangosteen pericarp [21]. Weibull model was selected based on higher R^2 value, lower values of chi-square (χ^2) and standard error of the mean (SEM) [21]. On the other hand, Costa et al. [12] revealed that thermal degradation of anthocyanins in acai-berry pulp followed first order model. They observed that first order model has the highest R^2 values among the linearized zero, first and second order investigated. Sarkis et al. [22] evaluated zero order, first order and Weibull models for good fit of experimental data for thermal degradation of anthocyanins in blackberry pulp. They omitted zero order due to higher values of chi-square, standard error of mean and average error. First order was used to model the results and obtain the degradation rate constant due to its mathematical simplicity, although both first order and Weibull models showed good fit with reasonably low values for chi-square, standard error of mean and average error [22].

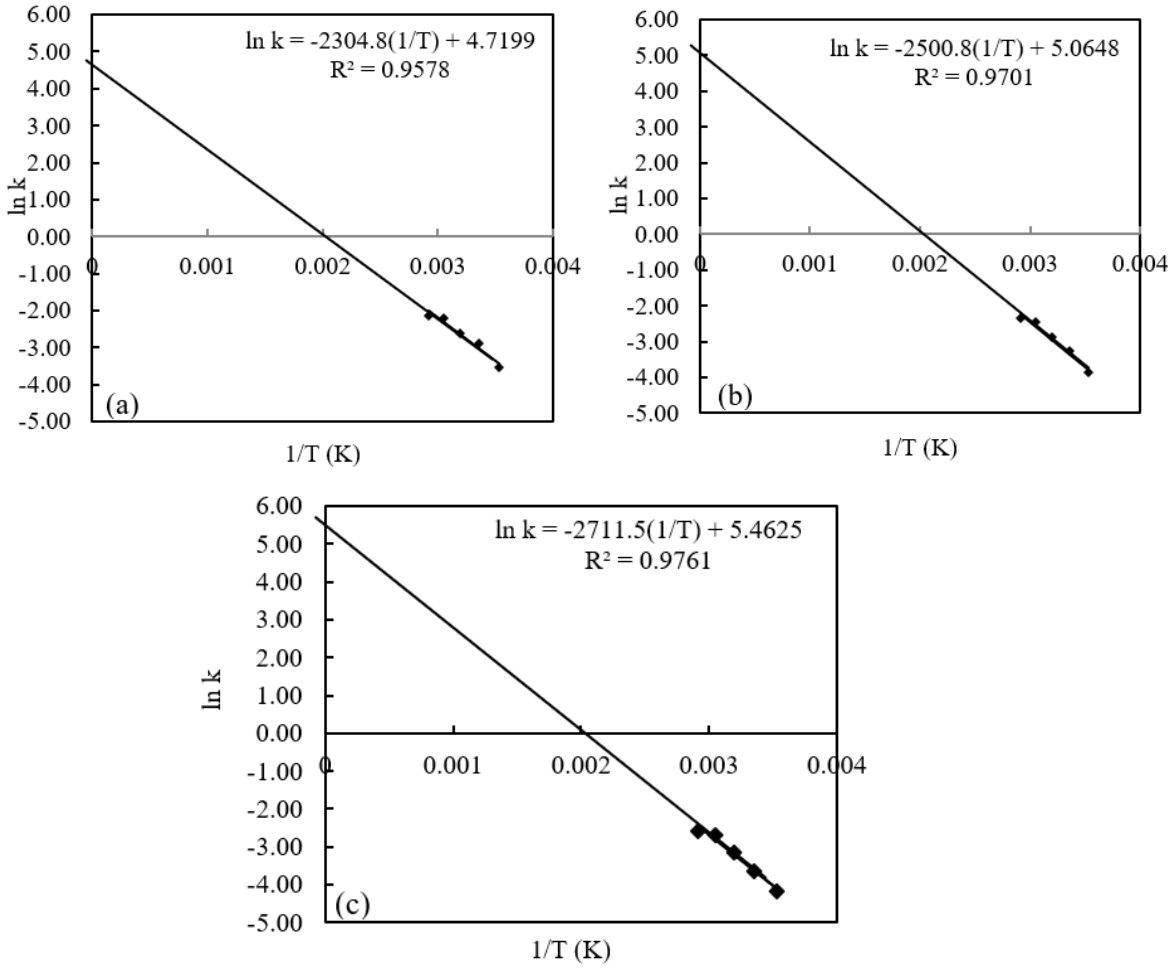

Fig. 4 (a) Zero-, (b) first-, and (c) second-order Arrhenius plots of temperature dependence of anthocyanin from *Garcinia Mangostana* L. rind.

Activation energy (E_a) and frequency factor (A) were calculated from the slope and interception of second-order model (Fig. 4c) as 22.54 kJ/mol and 235.69, respectively. A high E_a signifies that the rate constant depends strongly on temperature [19]. Hence, the higher the E_a indicates that the rate of thermal degradation reaction is more sensitive to temperature [19]. Table 1 shows activation energies of anthocyanins obtained from present work and previous studies. Worth noting that although from the same plant material of mangosteen peel, E_a obtained are varied (Table 1). Factors attributed to the difference in E_a values were most probably due to different range of temperature studied and kinetic model applied (Table 1). The range of temperature studied by Chisté et al. [6] was 5, 28, 40, and 50°C, and Deylami et al. [21] was 60, 70, 80, 90, and 100°C, while the present work was 10, 25, 40, 55, and 70°C. E_a values also varied with plant sources (Table 1).

Table 1 Activation energies obtained for anthocyanin derived from various plant materials.

Ref.	Model	Activation energy, E_a (kJ/mol)	Plant source
Present study	Second order	22.54	*Gacinia mangostana* L. rind extract
[5]	First order	61.51	Mangosteen peel extract
[23]	Weibull	57.66	Mangosteen pericarp
[7]	First order	75.70	Cherry pomace
[10]	First order	99.77	Juçara extract
[10]	First order	93.62	Italia grape extract
[11]	First order	46.32	Urmu mulberry concentrate
[12]	First order	24.16	Açai pulp
[13]	First order	66.37	Blueberry puree
[16]	First order	94.00	Blackberry juice
[17]	First order	58.55	Cornelian cherry crude extract

Half-life ($t_{1/2}$), the time needed for 50% degradation of anthocyanins at temperature of 10°C, 25°C, 40°C, 55°C and 70°C were calculated using second-order model (Eq. 7) and tabulated in Table 2. It is clear that half-life of mangosteen's anthocyanin decreases with increasing storage temperature. Half-life of anthocyanin in mangosteen extract was decreased by 45% when subjected into storage temperature from 10°C to 25°C.

Table 2 Effect of temperature on the half-lives of anthocyanins from *Garcinia Mangostana* L. rind.

Temperature (°C)	Half-life $t_{1/2}$ (h)
10	44.16
25	25.40
40	15.64
55	9.79
70	8.91

Many studies have discovered that half-life of anthocyanins decreases with increasing storage temperature, however, the degree of decrease is varied among the studies. Chisté et al. [6] reported the half-life times of the anthocyanins extract of mangosteen peel were 4006 h at 5°C, 370 h at 28°C, 125 h at 40°C and 93 h at 50°C, which showed a huge difference from present study. An approximately 90% decrease of half-life was observed when anthocyanins extract subjected from 5°C to 28°C [6], which is very much higher compared to the present study. The huge difference of half-life between Chisté et al. [6] and the present work was most probably due to different time interval of a sample taken out for anthocyanins measurement during storage. Sample was taken out every hour for anthocyanins determination for a duration of 4 hours in the present work. While in Chisté et al. [6]'s study, measurement of anthocyanins was carried out every 2 days at 5 and 28 °C and every day at 40 and 50°C storage temperature but did not mention the total storage duration. On the other hand, Costa et al. [12] observed that half-life of anthocyanin in açai-berry pulp was decreased from 28.6 to 10.7 h when temperature increased from 40°C to 80°C, which was approximately 62.59%. Kara and Erçelebi [11]

reported that half-life of anthocyanin in Urmu mulberry concentrate was decreased from 8.3 to 3.2 h when heating from 60°C to 80°C. Half-lives of anthocyanins in blueberry puree have been discovered decreasing from 346.6 to 38.7 min when temperature increased from 70 to 105°C [13].

Temperature quotients (Q_{10}) were calculated using Eq. 9 and tabulated in Table 3. Result shows that the Q_{10} values decreased from 10°C to 70°C temperature zone. The higher the Q_{10} values during storage, the more sensitive anthocyanins will be, in responding to the temperature elevation or variation during heating treatment [6]. Higher Q_{10} was obtained for lower storage temperature (10 to 25 °C) indicating anthocyanins derived from *Garcinia Mangostana* L. rind is more sensitive to temperature elevation at low storage temperature as compared to high temperature (Table 3).

Table 3 Temperature quotient (Q_{10}) of studied temperature zone with activation energy of 20.75.

Temperature zone (°C)	Q_{10}
10 to 25	1.446
25 to 40	1.382
40 to 55	1.367
55 to 70	1.065

This result is in agreement with previous studies [6,10]. Higher Q_{10} values of 4.4 and 3.5 were obtained at low storage temperature (50 – 70°C) for both juçara and Italia grape extracts, as compared to Q_{10} values of 1.7 and 1.9 in high temperature (70 – 90°C) [2]. Chisté et al. [6] reported higher Q_{10} value of 10.8 for anthocyanins from mangosteen peel at low storage temperature (5 – 28°C) compared to high temperature (40 – 50°C) in which the Q_{10} obtained was 1.3. On the contrary, there were studies reported lower Q_{10} value at low storage temperature compared to high temperature [13,17]. Moldavan and David [17] observed a lower Q_{10} value of 1.346 at low storage temperature (2 – 22°C) in comparison to high storage temperature (22 – 75°C) of Q_{10} value of 2.361 for anthocyanins in Cornelian cherry crude extract. Their result indicated that the anthocyanins were more sensitive to temperature elevation at high storage temperature compared to low storage temperature. Similarly, blueberry puree has been revealed having lower Q_{10} value of 1.85 at low storage temperature (70 – 80°C) as compared to high temperature (95 – 105°C) of Q_{10} value of 2.08 [13].

4 Conclusion

Anthocyanins of *Garcinia Mangostana* L. rind has been revealed yielded 62.83% significantly higher of TMA value of 1.18 ± 0.0758 mg cy-3-glu/g compared to without stirring (0.439 ± 0.0453 mg cy-3-gly/g). This finding proven that stirring of the sample mixture able to accelerate the extraction which favouring diffusion of the analytes from the bulk solution to the extractant phase. *Garcinia Mangostana* L. rind extract is recommended to store at 10 °C for a better preservation of TMA value as anthocyanins has been observed in great degradation by 14.84%, 19.44%, 23.97%, and 29.61% when storing at temperatures of 25°C, 40°C, 55°C and 70°C, respectively, but only 7.04% degraded at 10°C, for a duration of 4 hours. This result also indicated that 10°C storage condition is ideal for long term storage whereas 25°C storage condition is viable for short term storage. There were no statistical differences between zero-, first-, and second-order models for anthocyanins degradation using graphical integrated rate law plots. However, Arrhenius plot with high R^2 value of 0.9761 has further justified that second-order is the best model to describe thermal degradation of anthocyanins derived from *Garcinia Mangostana* L. rind from 10°C to 70°C. Activation energy (E_a) of anthocyanins thermal degradation was 22.54 kJ/mol and half-lives were from 44.16 to 8.91 hour in temperature range from 10 to 70°C.

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