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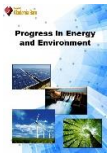

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Original Article

Anthocyanin degradation kinetics and thermodynamic analysis of *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L. and *Hibiscus sabdariffa* L.

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Abstract

Anthocyanins are natural occurrence red pigments existed in most flowers with high health benefited values. These anthocyanins rich flowers have a short shelf life and fast degradation when in fresh stage. Therefore, drying is a conventional way to preserve them from rotten in order to be reachable for urban consumers who have busy life style and limited space for planting. The present study was conducted to evaluate the anthocyanin degradation kinetics of *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L. and *Hibiscus sabdariffa* L. at drying temperatures of 50, 60, 70 and 80 °C for durations of 10, 20, 30 and 40 min. Anthocyanin degradation kinetic order of *Hibiscus rosa-sinensis L., Clitoria ternatea L. and Hibiscus sabdariffa L.* were determined by constructing natural logarithm Arrhenius equation plots from *k* values obtained from zero-, first-, and second-order integrated rate law plots at each temperature levels of 50 °C, 60 °C, 70 °C and 80 °C, based on the highest coefficient of determination (R^2). Fresh flower of *Clitoria ternatea* L. has been revealed possessed the highest amount of total monomeric anthocyanin (TMA) followed by *Hibiscus sabdariffa* L. and the *Hibiscus rosa-sinensis* L. Results revealed that anthocyanins degradation for *Hibiscus rosa-sinensis* L. and *Clitoria ternatea* L. followed first-order kinetic behaviour, while *Hibiscus Sabdariffa* L. followed the second-order. Anthocyanins of *Hibiscus Sabdariffa* L. has been discovered having high *k* values which led to shorter half-life values. However, anthocyanins of *Hibiscus Sabdariffa* L. is more stable during heat drying treatment as evidenced by higher activation energy (E_a) and activation enthalpy (ΔH), but lower free Gibbs energy (ΔG) and absolute value of entropy (ΔS) in comparison to *Hibiscus rosa-sinensis* L. and *Clitoria ternatea* L. Therefore, *Hibiscus sabdariffa* L. is highly recommended to be used as food colorant in food processing industries which involve heating.

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1 Introduction

The colourants used in food products are mainly classified as being natural colourant or synthetic colourant. Synthetic dyes can negatively affect the human body as they are poisonous to human body [1]. Currently, there has been various issues of allergy, toxicity and carcinogenic effects related to the use of synthetic dyes. Moreover, the binding of some organic material to the synthetic dye could also result in several harmful side effects to human. This is because the dye that is used for staining purposes are absorbed deeply into the material that is being stained through chemical bonds [2]. Natural pigments

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on the other hand not only gives colour to food products but also have various health promoting benefits [3]. One such pigment is the anthocyanins.

Anthocyanins are described as water-soluble pigments contained within the flowers and fruits of various plants [4]. The accumulation of anthocyanin plays a major role in determining the colour of the flower [5]. According to them, the higher the anthocyanin content, the darker the colour of the flower obtained. Several flowers have been proven to be rich in anthocyanin content. These include *Hibiscus rosa-sinensis* L. [6,7], *Clitoria ternatea* L. [8,9] and *Hibiscus sabdariffa* L. [10,11]. They have the ability to enhance the appearance of the flowers. They are used as natural colourants due to their availability in various colours [12]. Hence, they are gaining more attention for their use as natural food additives [13].

Hibiscus sabdariffa L. and *Hibiscus rosa-sinensis* L. are species of the *Malvaceae* family that can be found whole year round in tropical and subtropical regions of the world [14]. On the other hand, *Clitoria ternatea* L. is a member of the *Fabaceae* family that is found typically in tropical countries. This include Indo China, Madagascar and the Phillipines Islands [15]. The anthocyanins contained in plants like these are not only attractive due to their colour, but are also attractive due to their various health benefits. Anthocyanins possess properties of antioxidant [16,17], anti-diabetic [18], anti-inflammatory and artheroprotective [19], anti-tumoral [20] as well as antimicrobial and hepatoprotective [21]. Anthocyanins have been reported to be used in food products as well as in cosmetics [22]. Not only that, due to these beneficial reasons, these anthocyanins can also be consumed directly by people. The food industries are in great demand for high supply of natural colourants yearly [3]. There is a demand for new sources of anthocyanins that have high tinctorial power, highly available and low in cost [23]. Bringing back the use of natural dyes in foodstuffs has become a point of interest. This is because the natural dyes coming from various plant species not only have better biodegradability, but are also friendly towards the environment [2]. Since the use of natural dye instead of synthetic dye in food products is safer and better for human health [1], it is urged that food industries use natural colourants more than synthetic colourants [3].

In order to obtain the anthocyanins from the plant materials, drying must be performed to prolong the lifespan of the plant material or fruits [24]. Drying is one of the ancient methods used by humans for preservation of food. The moisture content of the food material is reduced tremendously which improves the preservation of the food products [25]. However, heat applied in drying process might result certain extent of anthocyanin degradation [26]. Thus, the present study was aimed to investigate the anthocyanin degradation kinetics of *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L. and *Hibiscus sabdariffa* L. at drying temperatures of 50, 60, 70 and 80 °C for durations of 10, 20, 30 and 40 min.

2 Materials and methods

2.1 Preparations of samples

The flowers of *Hibiscus rosa-sinensis* L. (Fig. 1a) and *Clitoria ternatea* L. (Fig. 1b) were harvested from a home garden in Kuala Lumpur, Malaysia and *Hibiscus sabdariffa* L. (Fig. 1c) flowers were bought from a local market in Bangsar, Kuala Lumpur, Malaysia. *Hibiscus sabdariffa* L., calyx, *Hibiscus rosa-sinensis* L. petals and *Clitoria ternatea* L. petals were removed from the flowers.

One gram of each of the samples were extracted using water as the solvent. 20 ml of water was used for the solvent. The extraction was performed at room temperature (25 ± 0.1 °C) with an extraction time of 30 minutes on a magnetic stirrer. This is to ensure that the extraction of anthocyanins occurred uniformly throughout the extraction process. Subsequently, the mixture was filtered through filter tea bag. The filtrate collected were used to determine the total monomeric anthocyanin (TMA) using pH-differential method.

2.2 Determination of total monomeric anthocyanins (TMA) using ph-differential method

The total monomeric anthocyanins (TMA) of the extracts was determined by using the method similar to Giusti and Wrolstad (2001). This method involved the use of UV-Visible Spectrophotometer (Model U-2900, Japan). Extracts were diluted with two buffer solutions of 0.025M potassium chloride at pH 1.0 and 0.4M sodium acetate at pH 4.5. The diluted extracts were allowed to reach equilibrium for a

period of 15 minutes. The measurements of absorbance value for each respective dilution extract was obtained at 510 nm and 700 nm. Measurement of absorbance at 700 nm was to account for haze. Distilled water was used as the blank. The total monomeric anthocyanin (TMA) in the sample was calculated using Eq. (1).

$$\text{TMA} = \frac{A \times MW \times DF \times 1000}{\varepsilon \times 1} \quad (1)$$

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, ε = 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 0.05g/ml.

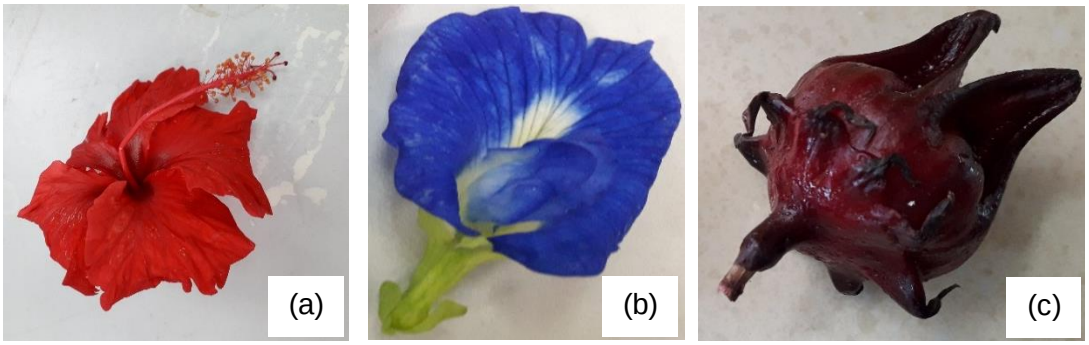


Fig. 1 (a) *Hibiscus rosa-sinensis* L, (b) *Clitoria ternatea* L., (c) *Hibiscus sabdariffa* L.

2.3 Evaluation effect of drying duration and temperature on anthocyanins degradation kinetics order and thermodynamic analysis

The petals of each flower were allowed to dry in a laboratory oven (Carbolite, Germany) for all combinations of four drying time (10, 20, 30, 40 min) and temperature (50, 60, 70, 80 °C). The dried samples were immediately wrapped in aluminium foil papers and stored in a dark area at room conditions to avoid exposure to light. These foil papers were labelled according to their respective drying time and temperature. The dried samples were then ground separately using a pestle and mortar to increase the surface area. For each different run, 1 g of the dried and ground samples were used to analyse anthocyanin content (TMA).

TMA determined at each drying time were plotted using zero- (Eq. 2), first- (Eq. 3), and second-order (Eq. 4) integrated rate laws at each temperature level [27].

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

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

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

where $(\text{TMA})_t$ = anthocyanin concentration (mg cy-3-glu/g) at time t; $(A)_0$, $\ln(A)_0$, and $1/(A)_0$ were intercepts of zero-, first- and second-order; k = reaction rate constant (min^{-1}). k values obtained from zero-, first-, and second-order integrated rate law plots at each temperature levels of 50 °C, 60 °C, 70 °C and 80 °C were used to construct natural logarithm Arrhenius equation plots (Eq. 5).

$$\ln k = -\frac{E_a}{R}\left(\frac{1}{T}\right) + \ln A \quad (5)$$

where E_a= activation energy (J/mol); R = 8.314 (J/mol.K); A = frequency factor; T = Temperature (K).

Degradation kinetic order of the anthocyanins was determined from straight line slope with the highest coefficient of determination (R^2) of natural logarithm Arrhenius equation plots.
Subsequently, activation energies and half-life (Eqs. 6, 7, and 8) were calculated based on the kinetic order determined [27].

Zero-order: $t_{1/2} = (\text{TMA})_0 / 2k$ (6)

First-order: $t_{1/2} = \ln 2 / k$ (7)

Second-order: $t_{1/2} = 1/k(\mathrm{TMA})_0$ (8)

where $t_{1/2}$ = half–life (min); $(TMA)_0$ = initial anthocyanin concentration (mg cy-3-glu/g). $t_{1/2}$ is defined as the time needed for anthocyanin degraded to 50% from the initial concentration.

Thermodynamic parameters of activation enthalpy, ΔH (kJ/mol) (Eq. 9), Gibbs free energy, ΔG (kJ/mol) (Eq. 10), and activation entropy, ΔS (J/mol.K) (Eq. 11) of anthocyanin for thermal degradation were estimated [28–30].

$$\Delta H = E_a - RT \quad (9)$$

$$\Delta G = -RT \ln\left[\frac{k \times h}{k_b \times T}\right] \quad (10)$$

$$\Delta S = \frac{\Delta H - \Delta G}{T} \quad (11)$$

where h is Plank's constant (6.62608×10^{-34} J.s) and k_b is Boltzman's constant (1.3807×10^{-23} J/K).

2.4 Evaluation of anthocyanins release from tea bag containing samples

The hot boiled water extraction was performed similar to the method used by Bolade et al. [31] with some modifications. Both *Hibiscus rosa-sinensis* L. (Fig. 2a) and *Hibiscus sabdariffa* L. (Fig. 2c) were dried at 50 °C for 20 min, while *Clitoria ternatea* L. (Fig. 2b) was also dried at the same temperature but for 30 min. These drying conditions were determined based on the level of dryness of flower with high TMA yield. Each dried flower was weighed at 0.5g, 1 g and 1.5g and placed respectively in tea bags and allowed to undergo hot boiled water immersion using 50 ml of water in randomized run using three level two factors (3^2) full factorial design. The immersion process was conducted at durations of 10, 20 and 30 minutes for each different mass of dried sample. The TMA of the water solution was then determined by pH-differential method as described in previous section.

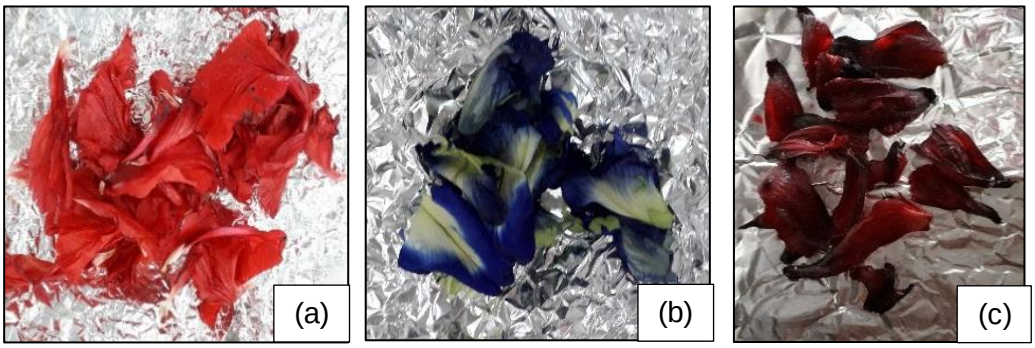


Fig. 2 Dried (a) *Hibiscus rosa-sinensis* L, (b) *Clitoria ternatea* L., (c) *Hibiscus sabdariffa* L.

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 = 6).

3 Results and discussion

3.1 Anthocyanins of fresh Hibiscus rosa-sinensis L., Clitoria ternatea L., and Hibiscus sabdariffa L.

Among the three flowers studied, *Clitoria ternatea* L. flower possessed the highest amount of TMA (0.6862 ± 0.0038 mg cy-3-glu/g), followed by *Hibiscus sabdariffa* L. with a TMA value of 0.6211 ± 0.00608 mg cy-3-glu/g and the *Hibiscus rosa-sinensis* L. flower yielded the least amount of TMA (0.6012 ± 0.0131 mg cy-3-glu/g) (Table 1). *Hibiscus rosa-sinensis* L. and *Hibiscus sabdariffa* L.

possessed similar TMA values ($p > 0.05$). Results from the present study is differed from previous work done by Siti Azima et al. [8]. They observed that *Clitoria ternatea* L. extract had the lowest TMA value as it was compared to fruits of *Ardisia colorata* var. *elliptica*, *Syzygium cumini* and *Garcinia mangostana* peel. TMA of fresh *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L. obtained from the present study are also compared with previous studies (Table 1). Although TMA value for *Clitoria ternatea L.* was the highest among the flowers studied, it is lower compared to previous studies done by Siti Azima et al. [8] and Phrueksanan et al. [9], where they reported 1.10 mg/g and 1.46 mg/g, respectively. The main factor attributed to TMA value difference was the extract preparation prior to pH-differential analysis. In Siti Azima et al. [8]'s work, *Clitoria ternatea* L. was stirred in distilled water for 10 min at 100 °C with ratio of plant parts to water of 1:4 (v/v). On the other hand, Phrueksanan et al. [9] boiled 0.5 kg of flower petals in 3 L of distilled water for 2 hours. While in the present study, 1 g of flower sample was extracted using 20 mL of water at room temperature for 30 min on a magnetic stirrer.

Apart from extract preparation, plant genotypes were also attributed to TMA value difference. Sukkhaeng et al. [10] reported that dark purple closed calyx has the highest TMA value of 1948.43 mg cy-3-glu/100g dry calyx among the 15 roselle genotypes studied.

Table 1 TMA yields of fresh *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., and *Hibiscus sabdariffa* L.

References	*Extraction solvent used*	*TMA yield*
	Hibiscus rosa-sinensis L.	
Present study	*Water extract*	*0.6012 ± 0.013 mg cy-3-glu/g*
Afify and Hassan [6]	80% ethanol	153.20 µg/g
Mak et al. [7]	Aqueous extract	205.76 mg c-3-gE/100g
	Ethanolic extract	155.28 mg c-3-gE/100g
	Clitoria ternatea L.	
Present study	*Water extract*	*0.6862 ± 0.004 mg cy-3-glu/g*
Siti Azima et al. [8]	HCl/water/ethanol solution	1.10 mg cy-3-glu/g
Phrueksanan et al. [9]	Water extract	1.46 mg cy-3-glu/g
	Hibiscus sabdariffa L.	
Present study	*Water extract*	*0.6211 ± 0.006 mg cy-3-glu/g*
Sukkhaeng et al. [10]	Water extract	1948.43 mg cy-3-glu/100g dry calyx
Jung et al. [11]	Water extract	5.24 mg cy-3-glu/L
	Ethanol extract	3.16 mg cy-3-glu/L

3.2 Anthocyanins degradation kinetic order and thermodynamic analysis

The anthocyanins degradation kinetic orders of *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L were evaluated at drying temperatures of 50, 60, 70, 80 °C for 10, 20, 30, and 40 min. Table 2 shows the coefficient of determination (R^2) values of natural logarithm Arrhenius plots for *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L. using *k* values obtained from zero- (Eq. 2), first- (Eq. 3), and second-order (Eq. 4) integrated rate law equations. Arrhenius plots with higher R^2 value indicated the best fit model to the experimental data. Results showed that anthocyanins degradation for *Hibiscus rosa-sinensis* L. ($R^2 = 0.9972$) and *Clitoria ternatea* L. ($R^2 = 0.9865$) followed first-order kinetic behaviour, while *Hibiscus Sabdariffa* L. ($R^2 = 0.9036$) followed the second-order.

Numerous previous studies have applied zero- and first-order models simultaneously to evaluate and predict anthocyanins degradation. The best fit model was determined by the higher value of coefficient of determination (R^2). Chisté et al. [32] investigated zero- and first-order models for the prediction of anthocyanins degradation of mangosteen peel under exposure of four different lights of ultraviolet, fluorescent, incandescent, and infrared. They revealed that first-order was the best kinetic model described the anthocyanins thermal degradation from the higher R^2 values obtained. Costa et. al. [33] investigated thermal degradation of anthocyanins in acai-berry pulp using zero-, first-, and second-

order models. They revealed that first-order model best fitted the experimental data which concluded from the higher R^2 values obtained. Furthermore, Ng and Cheok [34] evaluated degradation kinetic order of anthocyanins extracted from mangosteen rind using zero-, first-, and second-order models simultaneously. They found out that anthocyanins degradation of mangosteen rind was best described by second-order kinetic order for temperature range from 10 °C to 70 °C. They concluded the result based on the higher R^2 value of natural logarithm Arrhenius plots where the graphs were constructed using *k* values (rate of reaction) obtained from zero-, first-, and second-order integrated rate laws at each temperature level.

Table 2 R^2 values obtained from natural logarithm Arrhenius plots of zero-, first-, and second-order for *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L.

		R^2
***Hibiscus rosa-sinensis* L.**		
Zero order	$\ln k = 74.24\frac{1}{T} - 7.3449$	0.6668
First order	$\ln k = -2461.7\frac{1}{T} + 3.714$	0.9972
Second order	$\ln k = -5960.1\frac{1}{T} + 15.704$	0.9923
***Clitoria ternatea* L.**		
Zero order	$\ln k = -11.054\frac{1}{T} - 4.966$	0.0010
First order	$\ln k = -3194.1\frac{1}{T} + 5.729$	0.9865
Second order	$\ln k = -6731.4\frac{1}{T} + 17.653$	0.9783
***Hibiscus sabdariffa* L.**		
Zero order	$\ln k = 1091.8\frac{1}{T} + 8.4482$	0.8524
First order	$\ln k = -2549.8\frac{1}{T} + 3.9441$	0.8468
Second order	$\ln k = -6535.4\frac{1}{T} + 17.411$	0.9036

Although there were numerous studies on anthocyanins degradation kinetic, effect of drying conditions on anthocyanins degradation of *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L were rarely reported. First-order kinetic order has been commonly used to describe anthocyanins degradation. First-order kinetic model was used to describe anthocyanins degradation of temperature effect (60, 70, 80, 90, and 100 °C) on *C.ternatea* aqueous extract [35] and storage (3, 6, 9, and 12 days) on *Hibiscus sabdariffa* L. [36], respectively. Maciel et al. [37] used first-order kinetic model to describe thermal (30, 60, 70, 80, 90, and 100 °C) degradation of *Hibiscus sabdariffa* anthocyanins-rich extract with absence and presence of copigments of phytic acid dipotassium salt and chlorogenic acid.

Table 3 presents anthocyanins degradation rate constant (*k*), half-life ($t_{1/2}$), activation energy (E_a), activation enthalpy (ΔH), Gibbs free energy (ΔG) and activation entrophy (ΔS) for *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L. Although both anthocyanins in *Hibiscus rosa-sinensis* L., and *Clitoria ternatea* L. degraded with increasing temperature following first-order kinetic model, *Hibiscus rosa-sinensis* L. has higher *k* values as compared to *Clitoria ternatea* L. from 50 °C to 70 °C. This indicated that anthocyanins of *Hibiscus rosa-sinensis* L. is more thermal sensitive.

Among the three flower species, anthocyanins of *Hibiscus sabdariffa* L. has the fastest degradation rate as evidenced by the high *k* values obtained (Table 3). $t_{1/2}$ values obtained for *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L. from 50 °C to 80 °C were 18.24 to 34.48 min, 17.29 to 41.01 min, and 3.91 to 22.12 min, respectively (Table 3). These results indicated that the higher the *k* values, the shorter the $t_{1/2}$ values. This observation is in agreement with Qiu et al. [29] on anthocyanins in purple potato. They observed that the when *k* values increased from 0.67×10^{-2} to 1.07×10^{-2} min^{-1}, $t_{1/2}$ values decreased from 103.45 to 64.78 min, for drying from 50 to 85 °C. This study revealed that anthocyanins of *Hibiscus sabdariffa* L. has the highest activation energy (E_a) of 54.34 kJ/kmol, followed by *Clitoria ternatea* L. (26.56 kJ/kmol), and *Hibiscus rosa-sinensis* L. (20.47 kJ/mol). The higher the E_a indicates that the rate of thermal degradation reaction is more sensitive to temperature [27]. A high E_a also signifies that the rate constant depends strongly on temperature. Higher E_a indicates also higher energy is required to initiate the degradation reaction, thus, more stable the anthocyanins.

Table 3 Kinetic and thermodynamic parameters of anthocyanin of *Hibiscus rosa- sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L.

	Kinetic parameters		Thermodynamic parameters			
T (°C)	*k* (min^{-1})	$t_{1/2}$ (min)	E_a (kJ/mol)	ΔH (kJ/mol)	ΔG (kJ/mol)	ΔS (J/mol.K)
Hibiscus rosa-sinensis* L. *(First order)						
50	0.0201	34.48		17.78	100.81	-257.05
60	0.0250	27.73	20.47	17.70	103.41	-257.39
70	0.0320	21.66		17.61	105.90	-257.38
80	0.0380	18.24		17.53	108.56	-257.88
Clitoria ternatea* L. *(First order)						
50	0.0169	41.01		23.87	101.27	-239.64
60	0.0225	30.81		23.79	103.70	-239.98
70	0.0279	24.84	26.56	23.70	106.29	-240.77
80	0.0401	17.29		23.62	108.40	-240.18
Hibiscus sabdariffa* L. *(Second order)						
50	0.0728	22.12		51.65	97.35	-141.49
60	0.0882	18.25		51.57	99.92	-145.20
70	0.1576	10.22	54.34	51.48	101.35	-145.38
80	0.4120	3.91		51.40	101.57	-142.12

Activation energy (E_a) of anthocyanins degradation of *Hibiscus rosa-sinensis* L. is rarely investigated in previous study. However, E_a values for anthocyanins degradation for *Clitoria ternatea* L. and *Hibiscus sabdariffa* L. have been reported. Bragueto Escher et. al. [35] reported E_a value of 104 kJ/mol of anthocyanins degradation for *Clitoria ternatea* L. from 80 to 100 °C. The great difference in the value compared to the present study is most probably differed in anthocyanins quantification method employed and temperature range studied. Present study used pH-differential method while Bragueto Escher et al. [35] employed ultra-high-performance liquid chromatography to quantify anthocyanins content. On the other hand, Sinela et al. [38] reported that E_a of delphinidin 3-O-sambubioside and cyanidin 3-O-sambubioside from *Hibiscus sabdariffa* extract were 90 kJ/mol and 80 kJ/mol, respectively, in temperature range from 4 °C to 37 °C. Maciel et al. [37] reported E_a of 89.6 kJ/mol for anthocyanins derived from *Hibiscus sabdariffa* L. of temperature studied range from 31.8 °C (control) to 100 °C and anthocyanins were quantified at wavelength of 535 nm at pH1.0.

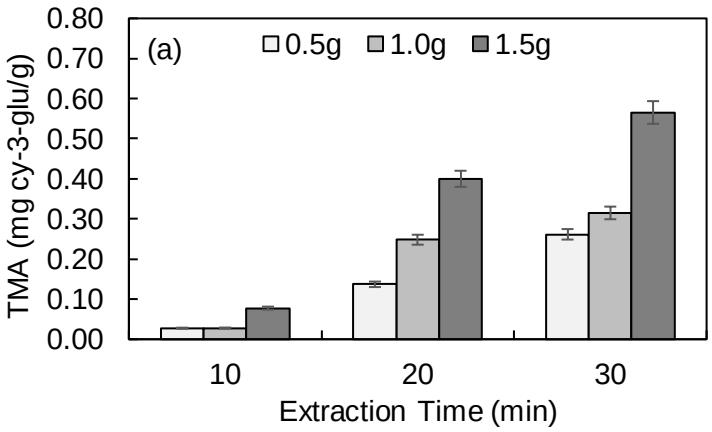
Esrimations of thermodynamic parameters of activation enthalpy (Δ*H*), Gibbs free energy (Δ*G*) and activation entrophy (Δ*S*) provide more information in explaining thermal degradation kinetics of anthocyanins derived from *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., *and Hibiscus sabdariffa* L. (Table 3). Activation enthalpy (Δ*H*) value is a measure of the energy difference between the reactant

and activated complex which associated to the strength of the chemical bonds in the formation for the transition state of reactant [28]. The formation of the activated complex is more easily occurred when ΔH value is small, because the potential energy barrier is low. Results showed that anthocyanins of *Hibiscus sabdariffa* L. has the highest ΔH (51.40 – 51.65 kJ/mol), followed by *Clitoria ternatea* L. (23.62 – 23.87 kJ/mol), and *Hibiscus rosa-sinensis* L. (17.53 – 17.78 kJ/mol) (Table 3). This indicated that larger energy potential barrier has to be overcome for anthocyanins of *Hibiscus sabdariffa* L. in order to transform to other states, which implied higher stability of its anthocyanins in comparison. Positive value of Δ*H* obtained in this study indicated anthocyanins degradation of these flower plants was an endothermic reaction [29]. The Gibbs free energy (Δ*G*) is an indication of the direction in which changes of food system will occur [29]. When there is no free energy difference (Δ*G*=0), the system is defined at equilibrium condition [29]. Hence, anthocyanins of *Hibiscus sabdariffa* L. is more stable as indicated from the slightly lower Δ*G* obtained (Table 3). The positive values of Δ*G* obtained for anthocyanins of the three flowers suggested that the anthocyanin degradations were nonspontaneous reactions [29,30]. Negative value of activation entrophy (Δ*S*) indicates sufficient energy for disorder change of molecules in the system [30]. The higher the absolute values of Δ*S* indicates the higher the difference of thermodynamic equilibrium of materials from the initial system [29]. This study revealed that anthocyanins of *Hibiscus sabdariffa* L. has lower range of absolute values of Δ*S* in comparison to *Hibiscus rosa-sinensis* L. and *Clitoria ternatea* L. (Table 3). This implied that anthocyanins of *Hibiscus sabdariffa* L. has higher resistance for disorder change of molecules due to lesser difference of thermodynamic equilibrium of materials from the initial system, in which led to more stability.

3.3 Anthocyanins release studies

Fig. 3 presents anthocyanins (TMA) release from tea bags containing 0.5 g, 1.0 g, and 1.5 g of dried *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., and *Hibiscus sabdariffa* L. in 50 mL of hot boiled water for immersion durations of 10, 20 and 30 min. Results show that the TMA content values in the water increased with increase in immersion time and solid mass within the tea bag, irrespective of flower types. It can be seen that for all three flowers, it was observed that the percentage increase of TMA content between 10 min and 20 min immersion duration was greater than the percentage increase of TMA content between extraction time of 20 min and 30 min. In terms of the yield of TMA obtained for the three different flowers, it can be seen that for all immersion conditions, the order of TMA yield obtained was *Clitoria ternatea* L. > *Hibiscus sabdariffa* L. > *Hibiscus rosa-sinensis* L. This shows that anthocyanins in *Clitoria ternatea* L. flower is more easily diffused out in comparison to *Hibiscus sabdariffa* L. and *Hibiscus rosa-sinensis* L.

These results are in accordance to that of Joseph and Adogbo [25] for their study on tea bag extraction of dried *Hibiscus sabdariffa* L. calyx using water. In their study, as the extraction time was increased for a fixed solid to solvent ratio, the concentration of diffused substance in the water increased. This is because the longer the time of extraction, the greater the diffusion of anthocyanins from the calyx into the solvent. Also, with an increase in weight of calyx for a fixed solvent volume, TMA yield increased. This simply means that with larger quantity of solid present, more extraction can occur which resulted in higher TMA yield.



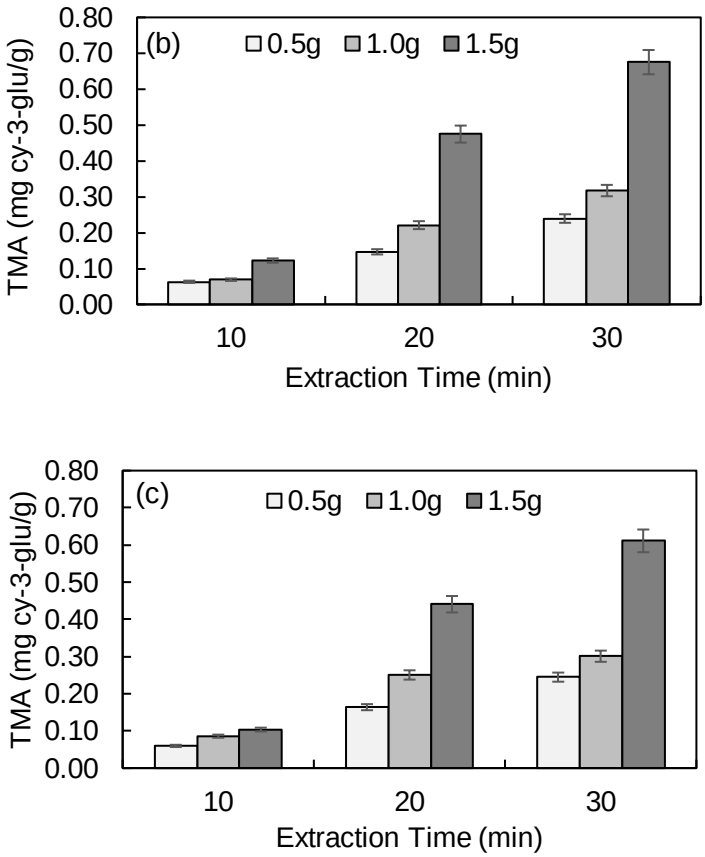


Fig. 3 Anthocyanins release from tea bags containing 0.5g, 1.0g, and 1.5 g of dried (a) *Hibiscus rosa-sinensis* L., (b) *Clitoria ternatea* L., *and* (c) *Hibiscus sabdariffa* L.

4 Conclusion

Fresh flower of *Clitoria ternatea* L. has been revealed possessing the highest amount of TMA followed by *Hibiscus Sabdariffa* L. and the *Hibiscus rosa-sinensis* L. Results showed that anthocyanins degradation for *Hibiscus rosa-sinensis* L. and *Clitoria ternatea* L. followed first-order kinetic behaviour, while *Hibiscus sabdariffa* L. followed the second-order using natural logarithm Arrhenius plots evaluated at drying temperatures of 50, 60, 70, 80 °C for 10, 20, 30, and 40 min. Anthocyanins of *Hibiscus sabdariffa* L. has been discovered the fastest degradation rate as evidenced by the high *k* values obtained. Half-life values obtained for *Hibiscus rosa-sinensis* L., *Clitoria ternatea* L., and *Hibiscus sabdariffa* L. from 50 °C to 80 °C were 18.24 to 34.48 min, 17.29 to 41.01 min, and 3.91 to 22.12 min, respectively. This study revealed that anthocyanins of *Hibiscus sabdariffa* L. is more stable during heat drying treatment as evidenced by higher activation energy (E_a) and activation enthalpy (ΔH), but lower free Gibbs energy (ΔG) and absolute value of entropy (ΔS) in comparison to *Hibiscus rosa-sinensis* L. and *Clitoria ternatea* L. Hence, *Hibiscus sabdariffa* L. is highly recommended to be used as food colorant in food industries which require heat treatment. On the other hand, for direct consumption of anthocyanin-rich drink prepared from the dried flowers, *Clitoria ternatea* L. flower is recommended. This is because the present study discovered *Clitoria ternatea* L is more easily diffused from tea bag which resulted higher anthocyanin content in 50 mL of hot boiled water as compared to *Hibiscus sabdariffa* L. and *Hibiscus rosa-sinensis* L., after immersion for durations of 10, 20 and 30 min. This study revealed that anthocyanins from *Hibiscus sabdariffa* L. is more suitable to be used in food industry. Nevertheless, an economic cycle and assessment should be performed before putting this anthocyanin for the industrial use.

Declaration of Conflict of Interest

The authors declared that there is no conflict of interest with any other party on the publication of the current work.

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