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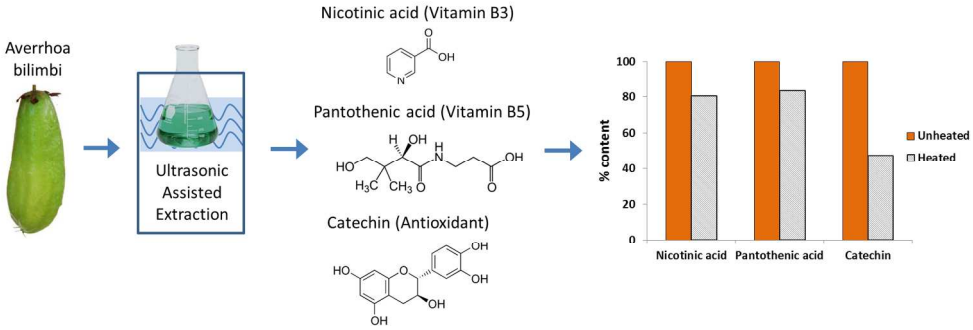

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Journal Name

ARTICLE

Thermal degradation kinetics of nicotinic acid, pantothenic acid and catechin derived from *Averrhoa bilimbi* fruits

Received 00th January 20xx,
Accepted 00th January 20xx

DOI: 10.1039/x0xx00000x

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Averrhoa bilimbi fruits contain appreciable amounts of polyphenolic compounds such as nicotinic acid, pantothenic acid and catechin, which can be recovered for use as food supplements. The recovery process of these polyphenols is often accomplished at slightly elevated temperatures, thus it is vital to understand the loss in polyphenolics due to thermal degradation before the mitigating method can be formulated. The thermal degradation kinetics of nicotinic acid, pantothenic acid and catechin derived from *Averrhoa bilimbi* fruits were investigated at temperature ranging from 90 to 120 °C and analysed using high performance liquid chromatography (HPLC). The results showed nicotinic acid, pantothenic acid and catechin degradation followed the first-order kinetics model. Pantothenic acid showed the lowest degradation rate constant, followed by nicotinic acid and catechin for all studied temperature, indicating a slowest degradation. The thermal degradation activation energy of nicotinic acid, pantothenic acid and catechin were 43.85 kJ mol^{-1}, 58.86 kJ mol^{-1}, and 21.27 kJ mol^{-1}, respectively. Pantothenic acid has the highest activation energy which implies that the compound is more sensitive to temperature change.

1. Introduction

Averrhoa bilimbi (vernacular name: 'belimbing buluh') belongs to the family Oxalidaceae. This plant is cultivated throughout tropical Asia and growing up to 15 m tall, with tree of around 30 cm in diameter.[1] *A. bilimbi* fruits are fairly cylindrical, 4-10 cm long with faintly five sided and produced in clusters. The fruits are very sour and used in the preparation of traditional dishes and production of pickles, vinegar and wine.[2] *A. bilimbi* is traditionally used in Malaysia for treatment of coughs, colds, itches, boils, pimples, scurvy, rheumatism, syphilis, diabetes and hypertension.[3] It is also used as antibacterial, antiscorbutic, astringent, post–partum protective medicine, treatment of fever, mumps and inflammation of the rectum.[3] These treatments are supported by a long history of human experience. Moreover, several in-vivo studies on the anti-hyperlipidemic and anti-diabetic properties of *A. bilimbi* extracts were reported earlier.[4,5] Previous scientific studies revealed that extract of *A. bilimbi* contained many useful bioactive compounds such as amino acids, citric acid, cyanidin–3–O–H–D–glucoside, phenolic, potassium ion, sugars and vitamin.[2,3] However, many phenolic compounds in *A. bilimbi* extracts are yet to be fully identified. Screening of phenolic compounds via ultra-high performance liquid chromatography quadrupole time of flight (UHPLC-QTOF) in this work has successfully identified a significant presence of nicotinic acid, pantothenic acid and catechin in *A. bilimbi* extracts. The presence of pantothenic acid and catechin was never reported previously for this fruit.

Nicotinic acid also known as vitamin B3 or niacin has been known for many years to pose antioxidant properties. Nicotinic acid has been used as vitamins and drugs. Numerous studies have reported the pharmacological effects and positive effect on diabetic patient.[6–8] Furthermore, nicotinic acid was reported to reduce cardiovascular risk and it has been very well documented that nicotinic acid could improve the cholesterol level.[9] Presence of nicotinic acid in *A. bilimbi* was reported earlier by Pushparaj et al.[10] and was further confirmed in this work.

Pantothenic acid is a water-soluble vitamin which is, also known as vitamin B5. It has beneficial effects on skin health and used in many pharmaceutical and cosmetic products for their skin emollient, regenerating and hair conditioning properties. It can be found in numerous commercial skin products (e.g., lip-sticks, aftershave lotions and hair preparations) owing to its regenerating, anti-inflammatory and anti-dermatitis properties.[11] Earlier work by Capodice[12] reported a significant reduction of global facial blemishes by the administration of a pantothenic acid-based dietary supplement. Besides, pantothenic acid is a required supplement for those who undergo regular dialysis treatment to alleviate the lipid abnormalities.[13] Moreover, pantothenic

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Electronic Supplementary Information (ESI) available: Compounds quantification by UHPLC-QTOF-MS. See DOI: 10.1039/x0xx00000x

acid has been widely used in fortified infant formulas because to helps bodies metabolize the macronutrients from milk.[14]

Catechin is a flavanol found in many plants and well-known as powerful natural antioxidant. Numerous studies on catechin have reported the pharmacological effects such as anti-hypertensive anti-diabetic and anti-inflammatory.[15,16] Earlier, Al-Hazzani & Alshatwi[17] reported that catechin has the potential for cervical cancer prevention and treatment. Thielecke & Boschmann[18] reported that catechin is useful for weight and body fat reduction. Most of these health-promoting properties are associated with antioxidant and free radical scavenging activities of catechins. Catechins have a hydroxyl group attached to its aromatic rings which act as an effective free radical scavenger (a powerful antioxidant). Therefore, catechin is a promising drug candidate for pharmaceutical and nutritional application.

Bioactive compounds such as vitamins, protein and antioxidant are known to suffer from a degradation process when exposed to high temperature over a long period, which is often the case during the extraction and powder making process. Many researchers studied the thermal degradation of other bioactive compounds such as vitamin C and vitamin A. For instance, Igual et al.[19] reported that flavonoids diminished markedly during application of thermal treatments but no significant differences in the rate of degradation between the different thermal techniques studied. Similar findings are also reported by Gamboa-Santos et al.[20] who studied the kinetics of vitamin degradation at different drying conditions. They reported heating time and temperature reduced the nutritive quality and sensorial evaluation of the product. Thermal degradation is undesirable because the degraded product is of low nutritional value and consequently, hampers the intention to produce a nutraceutical product.[21] Therefore, it is very important to understand the degradation kinetics of bioactive compounds from *A. bilimbi* to avoid excessive losses during product manufacturing. Thermal degradation kinetics of bioactive compounds from *A. bilimbi* extracts had never been studied before, and hence this is the aim of this work.

2. Experimental

2.1. Chemicals and plant material

Nicotinic acid, methanol, formic acid, HPLC grade acetonitrile was obtained from Merck (Darmstadt, Germany). Panthothenic acid standard was obtained from Supelco (Bellefonte, PA) and catechin standard was obtained from Fluka (AG, Switzerland). The fruit sample was collected from Kuantan, Malaysia. The pointed end of the fruit was removed and washed with excess water to remove impurities. The sample was cut into slices and stored at minus 80 °C before lyophilisation using a freeze dryer (Biotron, Cleanvac 12S, Korea). The lyophilised sample was ground into powder form with the particle size ranged from 2.8 to 893.4 µm and the volume weighted mean diameter [$D_{4,3}$] of 195 µm. The sample was kept at minus 20 °C prior to the extraction process.

2.2. Ultrasonic-assisted extraction (UAE)

Averrhoa bilimbi extracts were prepared using UAE in an ultrasonic bath (Crest P1800D Ultrasonics, Trenton, NJ) at 30 °C for 30 min at 144 W. Powdered *A. bilimbi* (1 g) was added to 8 ml of 50% methanol in a conical flask and immersed into the ultrasonic bath. Extracts were centrifuged (Eppendorf 5810R, Germany) at 1800 xg for 15 min and filtered using filter paper (Whatman No 1) to obtain a clear solution.

2.3. Heat treatments

Kinetics of thermal degradation of nicotinic acid, pantothenic acid and catechin in *A. bilimbi* extract were performed at three different temperatures, i.e., 90 °C, 100 °C and 120 °C. For comparison the degradation kinetics of pure compounds i.e., nicotinic acid, pantothenic acid and catechin at similar concentration as the fruit extract were studied at 90 °C. Sample (2 ml) was placed into a clean and dry screw cap stainless steel tube (outer diameter = 11 mm, inner diameter = 9 mm, length = 40 mm) to prevent evaporation. The samples were heated using an oil bath for times ranging from 1 to 40 min. Heated sample was cooled rapidly by plunging into iced water to stop further degradation. Unheated and filtered extract were used as a control sample.

2.4. High-performance liquid chromatography (HPLC) analysis

Quantification of nicotinic acid, pantothenic acid and catechin was performed using an Agilent 1200 series HPLC system (Agilent, San Jose, CA) equipped with an automatic injector, a column oven and a UV detector. Sample from the heat treatment experiment was separated using InertSustain C18 column (5 µm, 250 mm × 4.6 mm, GL Sciences Inc, Japan). The operating conditions were: injection volume, 20 µL; column temperature, 25 °C; flow rate, 1.0 mL min^{-1}. All samples and standards were filtered through a 0.22 µm nylon syringe filter. The mobile phase consisted of 0.1% formic acid in water (solution A) and 0.1% formic acid in acetonitrile (solution B). The elution scheme was: 0–20 min, 5–50% B; 20–23 min, 50–95% B; 23–24 min, 95-5% B; 24–25 min, 5% B. The detection wavelength for nicotinic acid was set at 254 nm, pantothenic acid at 197 nm whereas catechin was set at 280 nm.

2.5. Mathematical model for thermal degradation kinetics

The first-order model was employed to describe the thermal degradation of nicotinic acid, pantothenic acid and catechin according to the method reported elsewhere.[22–24] The first-order kinetics is given by the following equation:

$$[C(t)] = [C(0)]\exp(-kt) \quad (1)$$

where *C(t)* and *C(0)* are the concentration after heating time *t* and *t* = 0 min, respectively, and *k* is the reaction rate constant. The equation for first-order kinetics after integration of eqn (1) is given by:

$$\ln(C_t/C_0) = -kt \quad (2)$$

Half-lives ($t_{1/2}$) which is the time needed for 50% degradation was calculated as follows:

$$t_{1/2} = -ln\,0.\ 5/k_1 \quad (3)$$

where $t_{1/2}$ is the half-life and k_1 is the first-order degradation rate constant. The effect of temperature at constant pressure on the degradation rate constants is expressed by the Arrhenius equation:

$$k_T = A\exp(-E_a/RT) \quad (4)$$

where E_a is the activation energy of the degradation reaction (kJ mol^{-1}), R is the universal gas constant (8.3145 J mol^{-1} K^{-1}), T is the absolute temperature (K) and A is a pre-exponential factor. The reaction rate constant, k and activation energies, E_a were identified by linear regression in Microsoft Excel 2010.

2.6. Statistical analysis

Analysis of variance (ANOVA) of the triplicate data was performed by using the data analysis tools in Microsoft Excel 2010, and a least significant difference (LSD) test was used to compare the means with a confidence interval of 95%.

3. Results and discussion

3.1. Quantification of bioactive compounds in *A. bilimbi*

The quantitative analysis of nicotinic acid, pantothenic acid and catechin were performed using an Agilent 1200 series HPLC system. The concentration of the nicotinic acid, pantothenic acid and catechin were identified by comparing peak areas with the results of a calibration series using standards obtained from Merck, Supelco and Fluka, respectively. The seven point calibration curve shows good linearity of R^2 > 0.997. The compounds were identified by means of the retention time and UV spectra of the standard. UV spectra of nicotinic acid, pantothenic acid and catechin from *A. bilimbi* extracts were found to be good matches with the standards (Fig. 1). Further analysis using ultra-high performance liquid chromatography quadrupole time of flight mass spectrometry (UHPLC-QTOF MS) shows the presence of three compounds at 124.036 m z^{-1}, 218.1007 m z^{-1} and 289.0682 m z^{-1}, respectively (see supplemental data). Qualitative analysis and identification using Metlin database revealed their molecular weights belonged to nicotinic acid, pantothenic acid and catechin. The positive identification by retention time, UV spectra and mass spectra of the target compound in comparison with the external standards confirmed their presence in the extracts. Thus, the three identified compounds were used as the marker in this work. Fig. 2 show the HPLC chromatogram profile of *A. bilimbi* extracts during heat treatment. It shows that the retention time of the targeted compounds is not affected by the heat treatment, although the peak area reduced over time due to degradation. Therefore, HPLC analysis was employed for the remainder of this work for quantification of nicotinic acid, pantothenic acid and catechin content in the *A. bilimbi* extracts.

3.2. Thermal degradation of nicotinic acid, pantothenic acid and catechin in pure solutions and *A. bilimbi* extracts

Thermal stability of nicotinic acid, pantothenic acid and catechin in *A. bilimbi* extracts were studied at 90, 100 and 120 °C. Similar study using the standard was performed at 90 °C for comparison. Figs. 3 to 5 show the thermal degradation of nicotinic acid, pantothenic acid and catechin of the standard and *A. bilimbi* extracts as a function of time. The degradation of nicotinic acid, pantothenic acid and catechin increased with increasing heating temperature and time. The plot for nicotinic acid, pantothenic acid and catechin in the standard and *A. bilimbi* extracts shows a linear relation, which corresponds to first-order degradation kinetics with the correlation coefficient above 0.90 for all cases. Earlier, Nisha et al.[23] reported that nicotinic acid degradation in potato also follows the first-order kinetics at the temperature ranged from 50 to 120 °C. Other researcher also reported the similar finding.[24,25] Meanwhile, Gutzeit et al.[26] reported the first-order degradation of pantothenic acid derived from sea buckthorn berries. They studied the pantothenic acid content in juice and concentrate during processing and storage of sea buckthorn berries. Wang et al.[27] found that the thermal degradation kinetics of catechin in green tea also followed the first-order model at temperature ranging from 25 to 165 °C.

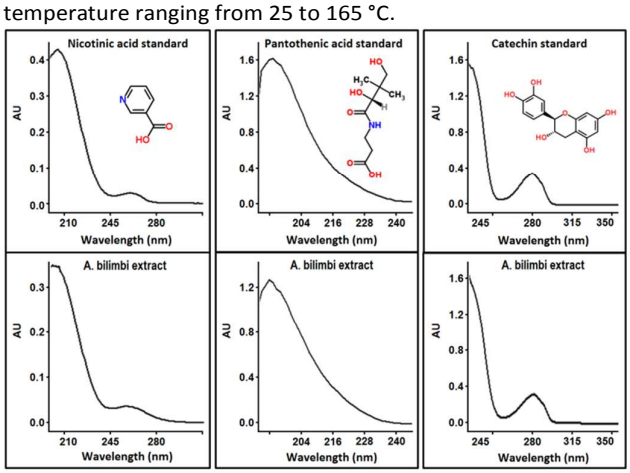

Fig. 1 Comparison of spectra for nicotinic acid, pantothenic acid and catechin from standard and *A. bilimbi* extract.

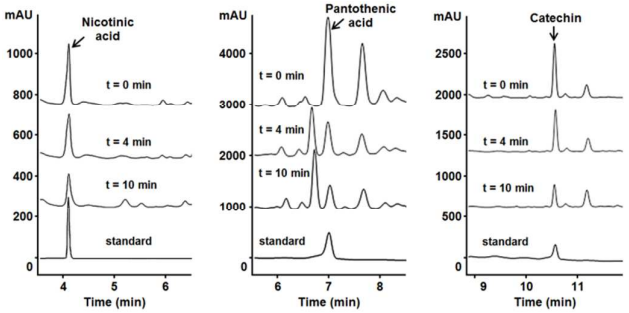

Fig. 2 HPLC chromatograph profile of *A. bilimbi* before and after heat treatment at 100 °C for 0, 4 and 10 min for nicotinic acid (recorded at 254 nm), pantothenic acid (recorded at 197 nm) and catechin (recorded at 280 nm).

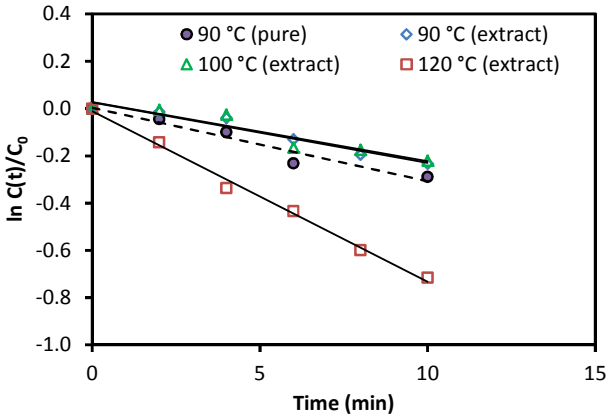

Fig. 3 Thermal degradation kinetics of nicotinic acid during heating at 90, 100 and 120 °C.

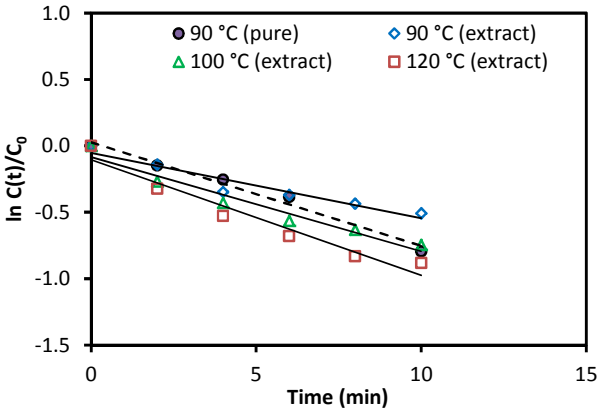

Fig. 5 Thermal degradation kinetics of catechin during heating at 90, 100 and 120 °C.

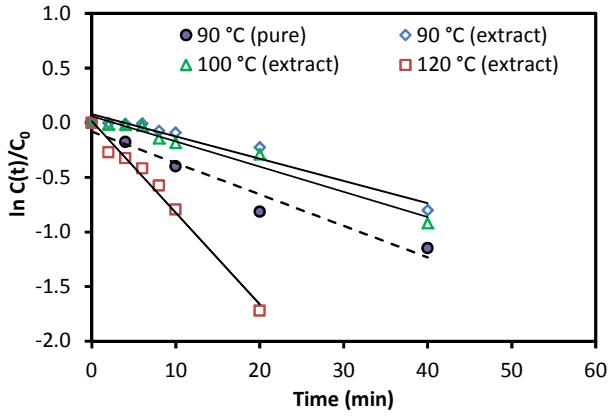

Fig. 4 Thermal degradation kinetics of pantothenic acid during heating at 90, 100 and 120 °C.

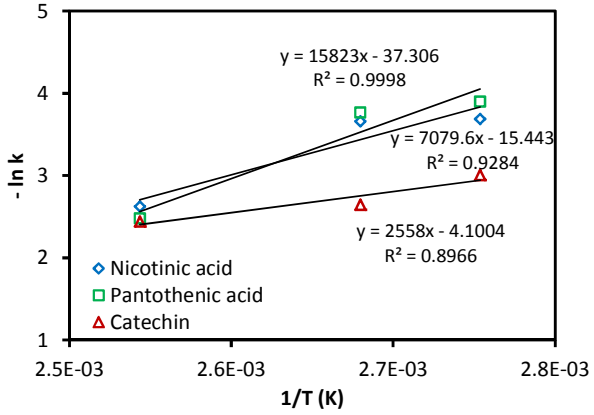

Fig. 6 Arrhenius plot for degradation of nicotinic acid, pantothenic acid and catechin in *A. bilimbi* during heating.

Table 1 Rate constant (k, min^{-1}) and half-life, $t_{1/2}$ of nicotinic acid, pantothenic acid and catechin in *A. bilimbi* at various temperature

Temp (°C)	Nicotinic acid		Pantothenic acid		Catechin	
	k (min^{-1})	$t_{1/2}$ (min)	k (min^{-1})	$t_{1/2}$ (min)	k (min^{-1})	$t_{1/2}$ (min)
90 (Pure)	0.0250	27.7	0.0203	34.1	0.0492	14.1
90 (Extract)	0.0311	22.3	0.0288	24.1	0.0779	8.9
100 (Extract)	0.0257	27.0	0.0231	30.0	0.0707	9.8
120 (Extract)	0.0722	9.6	0.0837	8.3	0.0869	8.0

The degradation rate constants, k, and half-life, $t_{1/2}$ for nicotinic acid, pantothenic acid and catechin in pure solution and *A. bilimbi* extracts is shown in Table 1. Degradation rate constant was estimated by the slope of the linearized plot of ln (C/C_0) versus t. The rate constant increased with temperature, suggesting that the degradations of all compounds are temperature-dependent. Comparison at 90 °C showed that degradation of nicotinic acid, pantothenic acid and catechin are faster in pure solutions compared to *A. bilimbi* extracts. This may be due to protective effects of some constituents in the extract on the degradation.[23]

Pantothenic acid showed the lowest degradation rate constant, followed by nicotinic acid and catechin. These results suggest that pantothenic acid is less prone to thermal degradation compared to nicotinic acid and catechin while catechin is the least heat-stable. The rate constant of nicotinic acid and pantothenic acid increased slightly when the temperature increased from 90 to 100 °C, but increased markedly by more than twofold at 120 °C. The results imply that nicotinic acid and pantothenic acid were highly unstable at 120 °C as confirmed by higher k values inferring a faster degradation. Finding from this work is consistent with the previously reported thermal stability of nicotinic acid and pantothenic acid.[23,28] Khalil and Mansour[29] also reported extensive degradation of nicotinic acid and pantothenic acid from faba beans when autoclaved at 121 °C. Nisha et al.[23] reported extensive degradation of nicotinic acid from potato as a result of cooking at 100°C in an open pan and 120 °C in

pressure cooker. Unlike the nicotinic acid and pantothenic acid, which showed a sudden change above 100 °C, catechin showed a consistent increase in degradation rate constants with temperature. This is due to the inherent thermolabile nature of catechin which can suffer from degradation even at ambient conditions.[30] Therefore, sudden change in *k* values for catechin was not observed for the range of temperature studied in this work.

The half-life value, $t_{1/2}$ for nicotinic acid, pantothenic acid and catechin decreased with increasing temperature (Table 1) due to the faster degradation rates at higher temperatures. At the same temperature, $t_{1/2}$ values for pantothenic acid were higher than that of nicotinic acid and catechin indicating higher thermal stability of the former.

Pantothenic acid is known as one of the most stable vitamins.[31] The stability of pantothenic acid is attributed to its aliphatic hydrocarbon structure, which has a higher thermal stability compared to the aromatic hydrocarbons found in both nicotinic acid and catechin. Higher number of aromatic rings in catechin structure resulted in lower stability as compared to nicotinic acid.[32] The stability of nicotinic acid and pantothenic acid is due to the presence of the carboxylic acid group to form two hydrogen bonds between a pair of molecules.[33] Furthermore, amide group and two methyl groups in the aliphatic chain of pantothenic acid contribute to its stability.[33] Meanwhile, the presence of two hydroxyl groups in catechin structure resulted in lower stability.[34] The hydroxyl group is known to be prone to thermal degradation.[35]

Figure 6 shows the Arrhenius plot of ln *k* against *1/T* for nicotinic acid, pantothenic acid and catechin in *A. bilimbi* extracts. The activation energy, E_a value for nicotinic acid was 43.85 kJ mol^{-1}, which is higher than the previously reported value (16.7 kJ mol^{-1}) at temperature 50 to 120 °C.[24] A higher activation energy implies that the compound is more sensitive to temperature changes, less stable and prone to degradation. According to Kubı et al.[36], presence of acids and alkaline solution assists the nicotinic acid hydrolysis. Since the present work was carried out at a slightly acidic condition (pH value of 4.5) which may affect the stability of nicotinic acid, and hence contributed to the difference in E_a value compared to previous study. The E_a value for pantothenic acid (58.86 kJ mol^{-1}) is higher than the previously reported value of 30.9 kJ mol^{-1} at 25 to 40 °C by Gutzeit et al.[24]. However, the previous work was performed at much lower temperatures, whereas the present work was performed at higher temperatures which may affect the activation energy values.[37] Higher temperature ranging from 90 to 120 °C in the present study may lead to higher activation energy of pantothenic acid. The E_a value for catechin (27.77 kJmol^{-1}) is lower than the previously reported value (41.6 kJmol^{-1}) by Li et al.[22] The difference of E_a value may be attributed to the different pH used in this work (4.5) as opposed to 5.1 in Li et al.[22] work. According to Wang et al.[38], catechin was found to be very stable at pH 3.0, however, become thermolabile at higher pH condition. Furthermore, Li et al.[22] obtained the E_a value at a much lower temperature ranging from 25 to 60 °C. As discussed earlier, temperature may affect the E_a value of the compound. Apart from pH and temperature, the difference in E_a values of nicotinic acid, pantothenic acid and catechin in the present study may be due to different soluble solid contents and compositional change in samples being treated.[39]

4. Conclusions

The presence of pantothenic acid and catechin in *A. bilimbi* fruit extracts was confirmed and reported for the first time. Thermal degradation kinetics of nicotinic acid, pantothenic acid and catechin in *A. bilimbi* extracts as well as in the pure solution followed the first-order model. The degradation rate of all three compounds increased as temperature and heating time increased. The degradation rates constant, *k* were of the sequence: pantothenic acid, nicotinic acid, catechin in the order of least sensitive to most sensitive. The activation energy, E_a for nicotinic acid, pantothenic acid and catechin in *A. bilimbi* extracts were 43.85 kJ mol^{-1}, 59.18 kJ mol^{-1} and 21.27 kJ mol^{-1}, respectively. The influence of temperature is more important for nicotinic acid and pantothenic acid due to its higher activation energy. Thermolabile compound such as catechin has a stable increase of degradation rate constant as oppose to a lower rate for both nicotinic acid and pantothenic acid below 100 °C. Although all compounds tested has about similar degradation rate constant at higher temperature (120 °C). The kinetics model established in this work may be useful to develop a processing method for *A. bilimbi* fruit that can preserve it bioactive content.

Conflicts of interest

The author declares no conflict of interest.

Acknowledgements

Financial support from Universiti Malaysia Pahang (GRS130315) is gratefully acknowledged. Norlia Muhamad acknowledges a scholarship from Ministry of Education, Malaysia.

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