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Research Article

Degradation kinetics and colour of anthocyanins in aqueous extracts of butterfly pea

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Abstract

The degradation kinetics and the colour display of anthocyanins in butterfly pea extract under different pH (1-10) and temperature (25, 50, 80 and 95ºC) were studied. First order thermal degradation parameters, such as *k*-values, activation energy (E_a), half-life values ($t_{1/2}$), *D*-values, *z*-values and Q_{10}-values, as well as the changes in Hunter lightness (L*), chroma (C*) and hue (H^o) were determined. Thermal degradation of anthocyanin in butterfly pea extract at pH 3 corresponds to first order kinetics. The Browning index, percentage polymeric colour, L* and H^0 parameters increased with time according to zero-order kinetics reaction (for L* and H^0), while C* decreased with time according to the first-order kinetics. For tinctorial strength, colourant concentration directly affected the L*, H^0 and C* values. The visual colour of the extract was also affected by tinctorial strength. The use of butterfly pea as a source of natural colourants shows potential use in the pH region of around 3.

Keywords: *Clitorea ternatea*, colourant, tinctorial strength, Malaysia

Introduction

The study of natural colourants is an active area of investigation due to the growing interest in substitution of synthetic colourants [1]. Anthocyanins are a group of compounds that give colour to plant pigment and are ubiquitous in the plant kingdom [2]. Anthocyanins are the largest water-soluble group responsible for the orange, red, and blue colours in fruit, vegetables, flowers, leaves, roots and other storage organs [3]. However, the use of anthocyanins as food colourants is still limited because of their relatively low stability during processing, formulation and storage. Anthocyanins are only used in acidic products where the pH is 4 or below. Colour intensity of anthocyanin is also pH dependent, the greatest is at pH 1 and decreasing rapidly as pH rises [4]. This characteristic limits the application of anthocyanins as a food colourant.

Bunga Telang or Butterfly Pea (*Clitoria ternatea*) is an example of a flower that contains anthocyanins and gives out the blue colour. Anthocyanins extracted from Butterfly Pea are stable in weakly acidic or neutral aqueous solution, hence its wide application as a food colourant in Southeast Asia [5]. Terahara *et al* [6], isolated six major anthocyanins ternatins (A1, A2, B1, B2,

D1 and D2), which were characterized as malonylated delphinidin 3,3',5'-triglucosides having 3',5'-side chains with alternating D-glucose and *p*-coumaric acid units [7]. Due to their high stability, anthocyanin extracts from Butterfly Pea flower are used to colour rice-cakes in Malaysia. The high stability is attributed to adsorption of the chromophores onto the starch of the glutinous rice [8].

This study focuses on the effect of pH and temperature on the stability and colour of aqueous anthocyanin extracts from Butterfly Pea. The effect of tinctorial strength on the visual colour of aqueous anthocyanin extracts from Butterfly Pea was also determined. This study will help to understand the effects of pH and temperature on the degradation kinetics and colour display of anthocyanin in Butterfly Pea extract in processing and storage and also to promote the use of Butterfly Pea as a natural colourant.

Materials and Methods

Preparation of anthocyanin extracts

The butterfly pea flowers were homogenized with nanopure water (1:50 w/v) followed by filtration through a tea cloth. The filtrate was cooled at 4^{o}C for 2 h prior to centrifuging at 4000 rpm, 2^{o}C for 30 min. The supernatant was collected and an aliquot of the extract was diluted at different pH buffers (Table 1). The sample and buffer ratio used was such that the visible maximum absorption of the extract at pH 1 was about 0.7. Similar ratio was used to prepare other pH solutions. Potassium sorbate (0.1% w/v) was added to all the anthocyanin solutions as a preservative. Final pH was measured and adjusted using either 6M NaOH or 4M HCl. The samples were allowed to equilibrate at room temperature in the buffer solutions for 1 h before measurements were taken. The aqueous anthocyanin extracts were transferred into a series of 25ml glass vials and stored at 25^{o}C in the dark.

Table 1: Preparation of 100 mL buffers (in nanopure water) at different pH values.

Buffer pH	Volume of required solutions	Volume of acid or base
1	25 ml of 0.2 M KCl	67ml of 0.2M HCl
2	25 ml of 0.2 M KCl	6.5ml of 0.2M HCl
3	50 ml of 0.1 M KHP	22.3ml of 0.1M HCl
4	50 ml of 0.1 M KHP	0.1ml of 0.1M HCl
5	50 ml of 0.1 M KHP	22.6ml of 0.1M NaOH
6	50 ml of 0.1 M KH_2PO_4	5.6ml of 0.1M NaOH
7	50 ml of 0.1 M KH_2PO_4	29.1ml of 0.1M NaOH
8	50 ml of 0.1 M KH_2PO_4	46.1ml of 0.1M NaOH
9	50 ml of 25 mM Borax	4.6ml of 0.1M HCl
10	50 ml of 25 mM Borax	18.3ml of 0.1M NaOH

Preparation of butterfly pea extracts at different tinctorial strengths (TS)
Butterfly pea flowers were homogenized in pH 1 buffer (1:50 w/v). The extracts were filtered and centrifuged at 4000rpm at 2^0C for 30 min. The supernatant was collected and adjusted to pH 3 using 6M NaOH. The anthocyanin extracts were transferred into 25ml glass vials and determined for TS using spectrophotometer and colorimeter. TS were calculated as the maximum absorbance in the visible range times the dilution factor [9]. In this study, we evaluated TS of aqueous anthocyanin extracts after 2 weeks storage at 25^0C.

Thermal stability and visual colour attributes of aqueous anthocyanin extracts
The samples were evaluated by measuring the changes in absorption at different wavelengths and comparing the colour retention or absorbance changes at maximum visible wavelengths. Measurement at 700 nm was taken as background correction. The effect of temperature on the stability and visual colour of the extracts was evaluated at pH 3, while thermal degradation was evaluated at 25, 50, 80 and 95°C after storing the extracts inside dark air-circulating ovens. Periodical measurements were carried out as follows; hourly (after 0, 2, 6 h), daily (after 1, 2, 3 days) and weekly (after 1, 2, 3, 4 weeks) for all extracts except for samples stored at 95°C where sampling was carried out at 0, 30, 60, 90 and 120 minutes.

Quantitative and qualitative analysis
Absorption spectra for Butterfly Pea extract was obtained between 400 – 700 nm using UV - spectrophotometer (Perkin Elmer Lambda 25 UV/VIS Spectrometer, United Kingdom). Colour changes were determined via the colorimeter test. The polymeric colour of extracts at pH 3 was determined according to Wrolstad [10], as follows; 0.2 ml of 20% $K_2O_5S_2$ was added to 2.8 ml of the extract. Then, measurement was taken at 420 nm, λ_{max} and at 700nm. A mixture between 0.2 ml of water and 2.8 ml extract acted as control. From the result, colour density was calculated as $[(ABS_{420} - ABS_{700}) + (ABS_{\lambda max} - ABS_{700})]$ x dilution factor (DF), polymeric colour as $[(ABS_{420} - ABS_{700}) + (ABS_{\lambda max} - ABS_{700})]$ x DF and browning index as $(ABS_{420} - ABS_{700}) / (ABS_{\lambda max} - ABS_{700})$. The percentage of polymeric colour was calculated as (Polymeric Colour/Colour Density) x 100.

First-order thermal degradation parameters were determined from the equation: $ABS/ABS_0 = e^{-kt}$. The temperature-dependant k-values were determined from the Arrhenius equation: $k_T = K_0e^{-Ea/RT}$. Half-life values were calculated as $t_{1/2} = \ln(2) / k_T$. D-values were calculated as $D = \ln(10) / k_T$. z-values were calculated by plotting log D vs. T. Q-values were calculated as $Q_{10} = 10^{10/z}$. The changes in L*, C* and Hº values were modelled according to zero-kinetics for the L* and Hº parameters (L* or Hº = kt) and according to first-order degradation kinetics for the C* parameters [11]. Statistics analysis and graphs were obtained using Microsoft Excel 2003 (Microsoft Corp., 2003).

Results and Discussion

Aqueous anthocyanin extracts at different pH values
Figures 1 and 2 show aqueous anthocyanin extracts at different pH values after 1 h of equilibration (Figure 1) and after 28 days of storage at 25ºC in the dark (Figure 2). pH 1 to 4 showed colour retention during the four weeks storage period at 25ºC in the dark. The colour for pH 5 to 10 faded to yellowish after one week. Extract at pH 5 and 6 became colourless after 3 weeks. The results of absorbance at day 1 and after 28 days for all pH conditions followed first order kinetics with $R^2 > 0.8357$. From the results (data not shown), percentage of absorbance changes at λ_{max} is higher at pH 1 (64.79%) with $R^2 = 0.9251$ while the lowest at pH 8 (17.61%) with $R^2 = 0.9993$. This clearly indicates that anthocyanin stability decreased with increasing pH values.

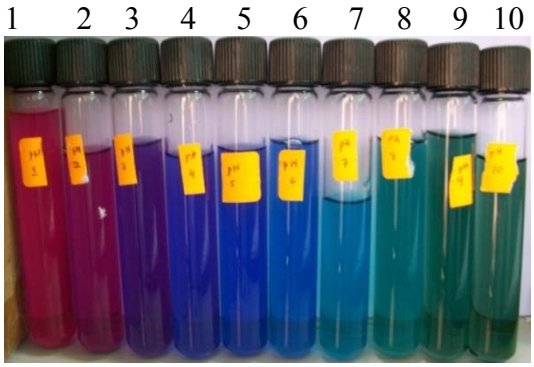

Figure 1. Anthocyanin extracts at different pH values after 1 h of equilibration.

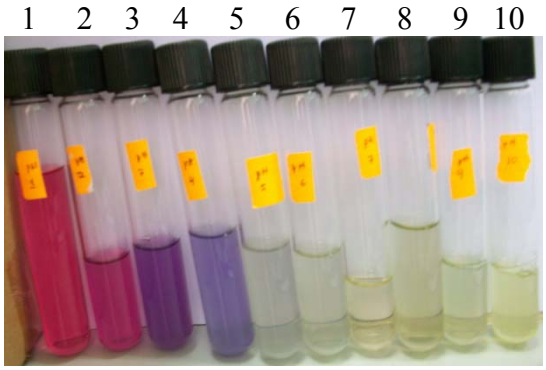

Figure 2. Anthocyanin extracts at different pH values after 28 days of storage at 25ºC in the dark.

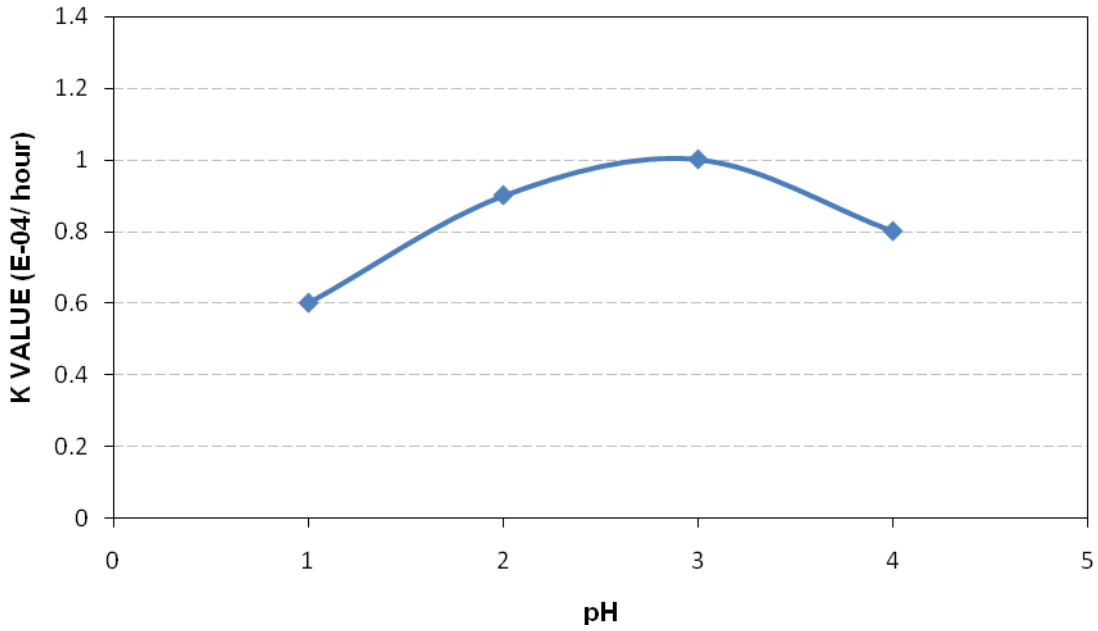

Figure 3. Effect of pH on the colour stability of aqueous anthocyanin extracts after 28 days of storage at 25ºC in the dark.

Figure 3 shows k-values for rate of reaction of aqueous anthocyanin extracts after 28 days of storage at different pH. pH 1 to 4 showed colour retention during the four weeks storage period at 25ºC in the dark, which is in agreement with earlier findings on the change in absorbance percentages of more than 45% compared to less than 45% for pH between 5 to 10. Therefore, further evaluation on the thermal stability of the aqueous extracts will be carried out at pH 3 since most food products are in the pH range from 3 to 7 [12].

Aqueous anthocyanin extracts at pH 3

Profiles for the thermal degradation of the anthocyanin extracts and the initial colorimeter and spectrophotometer measurements at pH 3 are shown in Tables 2 and 3 respectively.

Table 2. Results of thermal degradation parameters of aqueous anthocyanin extracts at pH 3.

Temp. (ºC)	k_T value (E^{-4}/h)[a]	D-value[b]	$t_{1/2}$[b]	z-value (ºC)	Q_{10}	Arrhenius equation	
						E_a (kJ/mol)[a]	K_0 (1/h)
25	9 (0.9790)	106.6 d	32.1 d	70.4	1.4	28.66 (0.9166)	99.5440
50	14 (0.9672)	68.5 d	20.6 d				
80	36 (0.9382)	26.7 d	8.0 d				
95	95 (0.9387)	10.1 d	3.0 d				

[a] Coefficient of determination (R^2) shown in parenthesis.
[b] Time units: h= hours; d= days.

Table 3. Initial colour attributes of aqueous anthocyanin extracts at pH 3.

Measurement	L*	C*	H^0	λ_{max}	% Polymeric colour	Browning Index
Reading	27.6	2.5	-13.1	573	20.14	0.36

Based on Table 3, thermal degradation of the anthocyanins extracts at pH 3 corresponded to the first order kinetics. The temperature dependence of thermal degradation values (k_T) was determined from the Arrhenius equation. The k_T values indicate that the k values are in descending order with decreasing temperature. Slower degradation occurs at 25ºC compared to 95ºC. The colour retention of anthocyanin extracts at 95ºC decreased with time (Figure 4) which is agreeable with the findings of Jackman and Smith [13]. Lower temperature gives higher half-life values as compared to higher temperature. Comparing the result obtained from this study with a similar study using purple carrot by Fernando *et al* [11], the activation energy (E_a) of butterfly pea is much lower (28.66 kJ/mol) than purple carrot (81.34 kJ/mol) while the z-value is higher (70.4ºC compared to 26.0ºC for purple carrot). The lower E_a and higher z-values can be associated with increased temperature dependence of the anthocyanins degradation rate.

In this study, anthocyanin degradation could have resulted in brown pigment formation and anthocyanin polymerization [11]. Browning index increased with time for 25, 50, 80 and decreased at 95ºC with the values of 1.06, 1.46, 3.52 and 0.40, respectively. The percentage polymeric colour also showed a similar trend with values of 29.99, 21.49, 70.25 and 23.77 respectively. Increased polymeric values were accompanied by losses in total monomeric anthocyanins. It is envisaged that anthocyanins were extensively polymerized during storage [14] which may be due of to several factors including residual enzyme activity or condensation reaction of anthocyanins with other phenolics [15].

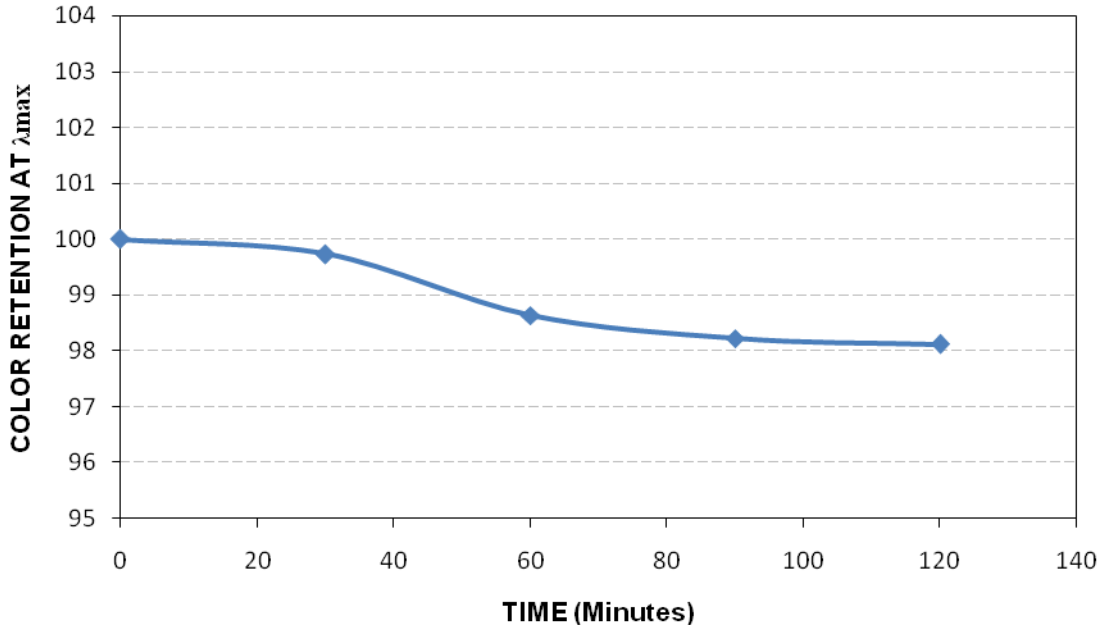

Figure 4. Colour retention of aqueous anthocyanin extracts stored at 95ºC in the dark.

Changes in visual color attributes

Results showed that lightness, L* and hue, H^0 (Figure 5) increased with time and followed zero-order kinetics reaction while chroma, C* (Figure 5) decreased with time and followed first-order kinetics. The increase in L* and H^0 values can be attributed to the formation of lightness or translucent extracts (due to the colour fading) and the formation of yellow chalcone species respectively, while the decrease in C* values would be related to the degradation of monomeric anthocynins or colour saturation [11]. The temperature dependence of k_{L^*}, k_{H^o} and k_{C^*} was determined from the Arrhenius equation as shown in Tables 4, 5 and 6. Lower temperature gives a higher half-life compared to higher temperature for the chroma parameters.

Table 4. Results of thermal degradation parameters for the lightness of aqueous anthocyanin extracts at pH 3.

Temperature (ºC)	Lightness	Arrhenius Equation	
	k_{L^*} (1/h)[a]	E_a (kJ/mol)[a]	K_0 (1/h)
25	0.0021 (0.8611)	18.9164 (0.5158)	3.8271
50	0.0022 (0.9232)		
80	0.0025 (0.9129)		
95	0.0142(0.9720)		

[a] Coefficient of determination (R^2) shown in parenthesis.

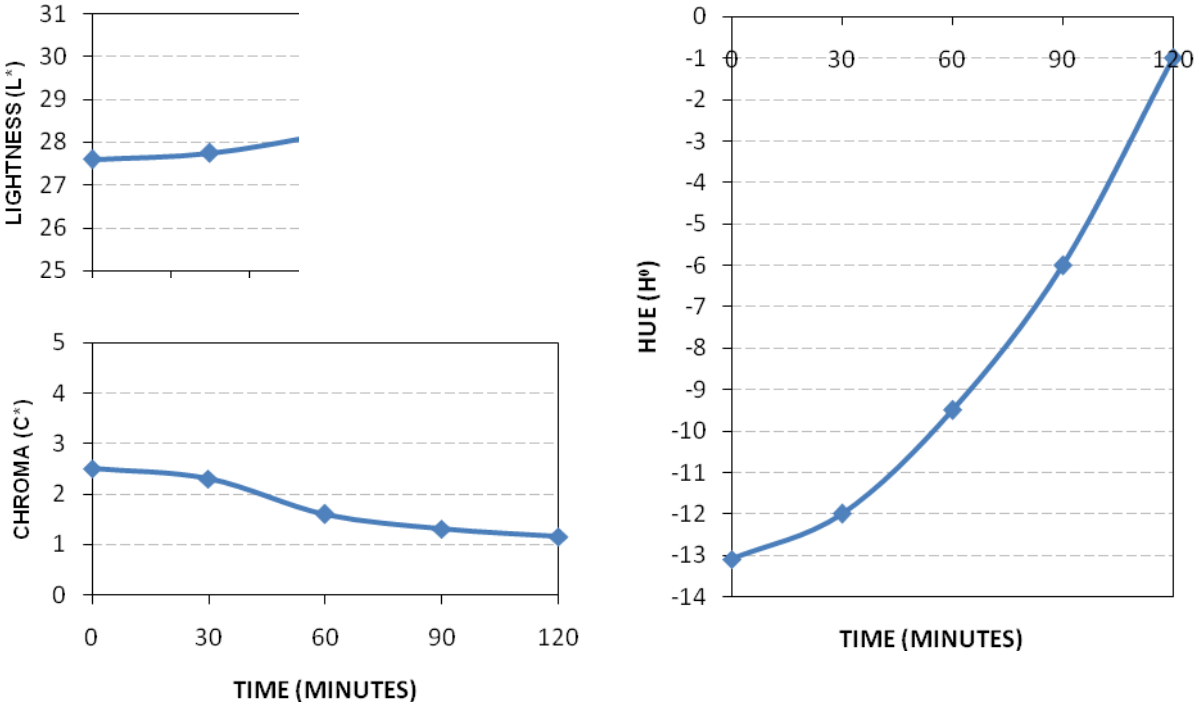

Figure 5. Hunter lightness (L*), hue (H°) and chroma (C*) parameters of aqueous anthocyanin extracts at pH 3 stored at 95°C in the dark.
Negative H° values were calculated as H° – 360°.

Table 5. Results of thermal degradation parameters for the hue of aqueous anthocyanin extracts at pH 3.

Temperature (°C)	Hue	Arrhenius Equation	
	$k_{H°}$ (1/h)[a]	E_a (kJ/mol)[a]	K_0 (1/h)
25	0.0874 (0.8574)	1.9505 (0.9747)	0.1944
50	0.0914 (0.8816)		
80	0.0998 (0.9354)		
95	0.1008 (0.8295)		

[a] Coefficient of determination (R^2) shown in parenthesis.

Table 6. Results of thermal degradation parameters for the chroma of aqueous anthocyanin extracts at pH 3.

Temp. (°C)	k_{C*} value (E^{-2}/h)[a]	D-value[b]	$t_{1/2}$[b]	z-value (°C)	Q_{10}	Arrhenius equation	
						E_a (kJ/mol)[a]	K_0 (1/h)
25	0.05 (0.7443)	191.9 d	57.8 d	30.5	2.1	64.99 (0.5424)	9.36E + 7
50	0.09 (0.8327)	106.6 d	32.1 d				
80	0.11 (0.9370)	87.2 d	26.3 d				
95	36.7 (0.9847)	6.3 h	1.9 h				

[a] Coefficient of determination (R^2) shown in parenthesis.
[b] Time units: h= hours; d= days.

Effect of tinctorial strength (TS)

After 14 days storage of the aqueous anthocyanin extract at 25ºC in the dark, the L* and C* (Figure 6) values increases while the H^0 (Figure 6) decreased sharply, in relation to colourant concentration. Therefore, the visual colour of butterfly pea extract was influenced by tinctorial strength. This could be due to the chemical structure of the anthocyanin. Their chemical structure governs colour, tinctorial strength and stability. Substitution can affect the tertiary and quartenary formations of the molecule, making the chromophore more or less susceptible to hydration, which causes colour loss [16].

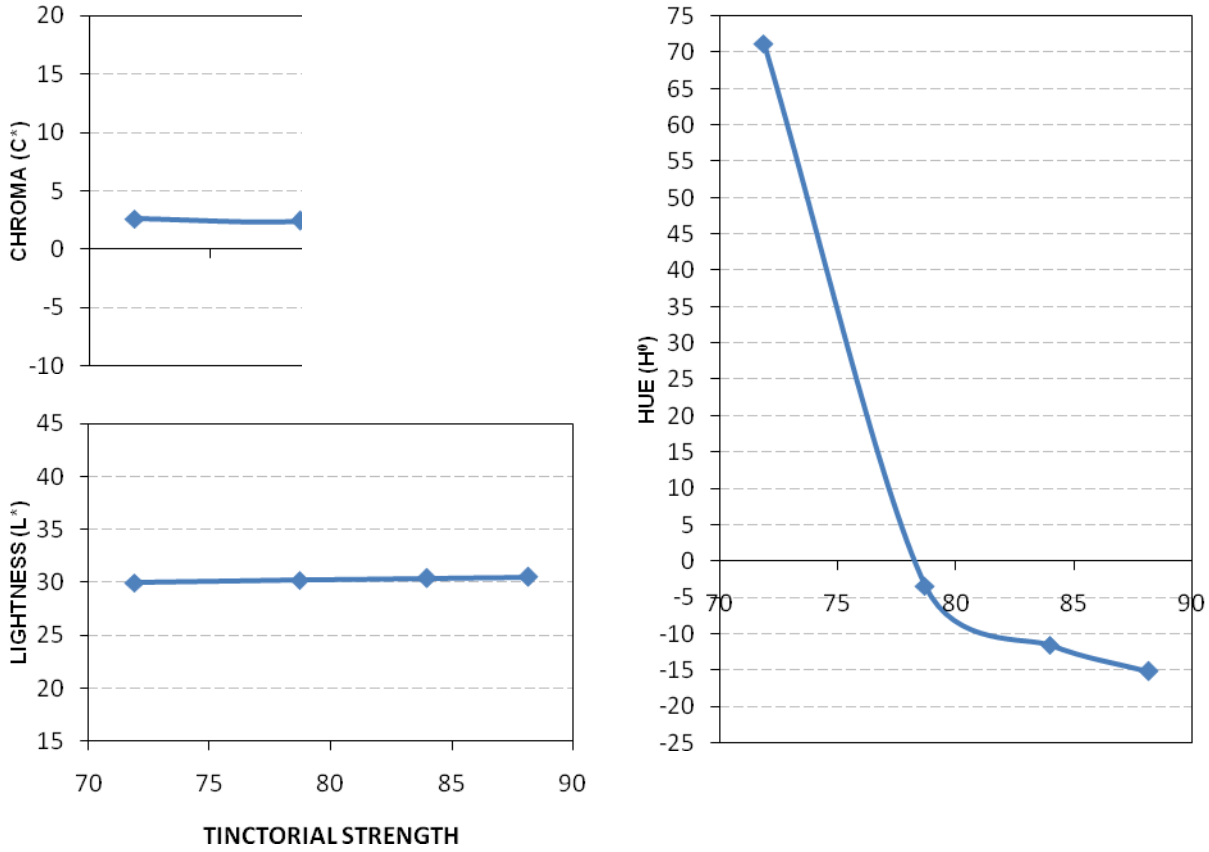

Figure 6. Effect of tinctorial strength on the Hunter lightness (L*), hue (H^0) and chroma (C*) parameters of aqueous butterfly pea anthocyanin extracts at pH 3.

Conclusions

The results show that stability of anthocyanins in aqueous butterfly pea extract is influenced by pH and temperature, while visual colour display is markedly affected by tinctorial strength. Thermal degradation of anthocyanin in butterfly pea extract at pH 3 corresponded to first order kinetics. The application of colourant from butterfly pea in aqueous environment should be done at relatively low temperature (i.e. room temperature) to avoid rapid changes in visual colour display. This study would help the potential use of butterfly pea as a source of natural colourants for the food industry and other technology by predicting the degradation changes of aqueous anthocyanin extracts from butterfly pea under different storage temperatures and periods.

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