

The Anthocyanins Content, Colour Changes and Thermal Stability of Roselle (*Hibiscus sabdariffa* L.) Petal Extract

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Abstract: *The objectives of this study were to determine the effect of heating treatment on anthocyanin stability and discoloration and to determine the degradation kinetics of anthocyanins and color of rosella petal extract. The results showed that temperature and heating time significantly affected on anthocyanin content, discoloration, and stability of anthocyanin extract of roselle petals. Thermal degradation of anthocyanin and color (a^* and C^*) decreased, while L^* , b^* , H^o , and ΔE increased along with the increase of temperature and time. In addition, the results of the study also showed that higher temperatures and longer storage caused a faster rate of degradation (higher k -value) and the shelf life ($t_{1/2}$) were shorter. It was clearly seen that anthocyanins were more stable at higher temperature with shorter time compared to heating at lower temperature in longer time.*

Keywords: roselle, anthocyanin, kinetics, discoloration, thermally

1. Introduction

Anthocyanin is a subgroup of flavonoids in plants that are mostly in the form of glycosides as anthocyanidins[1]. This dominant compound can be found in roselle petals especially which can cause a purplish red color. Anthocyanins are commonly used in the food industry as antioxidants and synthetic dyes substitutes for their bright and attractive colors and high solubility in water, making it possible to be applied to food systems[2-3]also. Anthocyanin as natural pigments that are susceptible to loss of color during storage[4]. Anthocyanin compounds can capture and neutralize free radicals in the body and food, so it is beneficial to health and can prevent food spoilage, but its application is often limited due to its low chemical stability[2,5-6]. The color and stability of anthocyanins are related to the presence of multiple double bonds in their structure. It can be said that structure is the cause of anthocyanin instability[7]. Even[8] also stated that anthocyanins have very unstable structures in both plant tissue and food products. Also[9], said that anthocyanins can be degraded through several processes that occur both during the extraction, processing, and storage of food. According to[9-10] anthocyanin, the damage is affected by temperature, pH, light, oxygen and enzyme factors.

[8]Based on its structure, anthocyanin is one of the most unstable pigments in food. Included in the anthocyanin pigment food industry also has low stability both when still is in the plant tissue as well as in the processed products. Several important chemical and physical factors have been involved in this anthocyanin degradation. According to [10], there are several things that can affect the stability of anthocyanins, among others, enzymatically and non-enzymatic reaction. Enzymatically, the presence of polyphenol oxidase enzymes affects the stability of anthocyanins. Factors affecting non-enzymatic anthocyanin stability are pH, light, temperature, and oxygen. As[11] said,

that anthocyanin is very sensitive to temperature, oxidation, pH, and light. Anthocyanins can be degraded through several processes that occur during the extraction, processing, and storage of food [9].

Other factors that are also important in anthocyanin stability include that must be processed and stored at low temperatures as well as in low availability of O₂, and avoided light. It has been observed that the anthocyanin color loss is the result of adding water molecule to C₂ from the flavylumcation, thus converting it to colorless hemiasetal[7]. Meanwhile, according to[12], the solution of anthocyanin extract with or without heat treatment showed excellent color and chemical stability at 4°C storage temperature.

The important content in roselle petals is the anthocyanin pigment that forms the flavonoids that act as antioxidants. Flavonoid rosella consists of flavanols and anthocyanin pigments. These anthocyanin pigments form an attractive reddish purple color in rosella petals. This plant as a tropical herbaceous plant spreads widely in Southeast Asia, West Indies, India, and many regions of Central Africa, West Africa, and America [13]. The uniqueness of Roselle is a sour taste in its refreshing petals because it has dominant acid compound namely ascorbic acid [14]. Acid is a compound that acts as an antibacterial and also gives the flavor of the product as a whole [15]. From the description above, we are interested to know the extent of anthocyanin stability and color changes during heating and storage at various temperatures.

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2. Experimental Section

2.1 Materials and Sample Preparation

Rosella fresh petals are obtained from the Pontianak city, West Kalimantan, Indonesia. Citric acid, potassium chloride, sodium acetate were analytical grade from Sigma-Aldrich.

Fresh rosella petals dried for 10 days followed by freeze-drying using freeze-dried (Freeze dryer, Alpha 1-2 LD) for 24 hours and mashed using a blender (Cosmos). The roselle petal powder was extracted using an aqueous acid solvent (citric acid 2%)[16] at a ratio of 1: 10 and macerated in a dark room at 4 °C for 24 hours, then filtered (the same treatment was repeated once again). The resulting extract was concentrated with a rotary evaporator (Laborota 4000, CVC 3000 vacuum pump) at 45 °C for 5 hours, after which it was stored at -18 °C until subsequent use. For the measurement of anthocyanin and color stability, the extract was diluted with distilled water (7.92g/1000mL).

2.2 Degradation studies

A 20 ml of the diluted extract were inserted into Erlenmeyer glass (50 mL). The Erlenmeyer glass was well sealed and put in a thermostatic shaking water bath (Julaba SW-22) with temperatures of 70, 80, 90, and 99.50°C observed at 20, 40, 60, 80 and 100 minutes. After heating treatment and time according to predetermined intervals, then the sample was immediately cooled in ice water. The temperature of the water bath was verified using a digital thermometer (Lutron: TM-946). An untreated sample was taken as a control and stored at 4°C.

A 15 ml of the diluted extract were inserted into a screw-tub tube (20 ml) then well sealed and placed in an incubator (Eyela Natural sterilizer NDS-601D) with temperatures of 30, 40, 50, 60, and 70°C and observed at 7, 14, 21, and 28 days.

2.3 Determination of anthocyanin content

Determination of total anthocyanin monomer used pH-differential method[17]. Measurement of the absorbance of each solution was done at λ 510 and 700 nm wavelengths, with buffer pH 1 and buffer pH 4.5 as the blank using spectrophotometer (Thermo Scientific Genesis 10S-UV-VIS). Calculation of the absorbance of the dissolved sample (A) as follows :

$$A = (A_{510} - A_{700})_{\text{pH}1.0} - (A_{510} - A_{700})_{\text{pH}4.5} \quad (1)$$

The anthocyanin concentration of the monomer pigment in the sample was calculated using the following formula:

$$\text{Anthocyanin monomer pigment (mg/liter)} = (A \times \text{MW} \times \text{DF} \times 1000) / (\epsilon \times L) \quad (2)$$

where:

MW : molecular weight of cyanidin-3-glucoside

449.2 g/mol

DF : dilution factor

ϵ : molar absorptivity Cyanidin-3- glucoside = 26900 L / (mol.cm)

L : Width cuvette = 1 cm

2.4 Color changes measurement

Color was measured using Colorimetric (Minolta type CR-400, Konica Minolta Co. Ltd, Osaka, Japan). The coordinates of L^* , C^* , H° were calculated from L^* , a^* , and b^* values based on the following equations[18-19]:

$$C^* = \sqrt{a^{*2} + b^{*2}} \quad (3)$$

$$H^\circ = \tan^{-1} b^*/a^* \quad (4)$$

To understand color changes, total color difference (ΔE) was computed using the formula[20]:

$$\Delta E = \sqrt{(\Delta L^* + \Delta a^* + \Delta b^*)^2} \quad (5)$$

2.5 Visual observation

A 15 mL sample was placed in a 20 mL screw-cap tube and a photographic image taken using a digital camera (Nikon D-3100 DSLR).

2.6 Kinetics anthocyanin degradation and color loss

The degradation kinetics of most biological materials and color changes of food system follow the zero-order equation reaction [21]:

$$\text{Zero order : } Ct = C_0 + k_0t \quad (6)$$

$$\text{First order : } Ct = C_0 \exp(k_1t) \quad (7)$$

where Ct and C_0 are the anthocyanin content at time t and t_0 , respectively, k_0 and k_1 are the zero- and first-order kinetic constants, respectively, and t is the storage time (minute or day).

The half-life time ($t_{1/2}$) from a zero order reaction and the first order was calculated using equation[22-24]:

$$T_{1/2} (\text{Zero order}) = C_0 / (2k) \quad (8)$$

$$T_{1/2} (\text{First order}) = \ln(2) / k \quad (9)$$

Dependence of the degradation rate constant k on temperature was quantified using the Arrhenius equation :

$$k = k_0 \exp\left(-\frac{E_a}{RT}\right) \quad (10)$$

where k_0 is the frequency factor (per min), E_a is the activation energy (kJ/mol), R is the universal gas constant [8.314 J/(mol·K)], and T is the absolute temperature (in degrees Kelvin, K). The Arrhenius activation energy (E_a) was calculated by plotting $-\ln(k)$ against $1/T$ (absolute temperature in Kelvin).

2.7 Data analysis

All experiments were carried out in triplicate and expressed as mean \pm SD. Kinetic data were analyzed using regression analysis with Microsoft Excel 2013 software (Microsoft Corporation).

3. Results and Discussion

3.1 The effect of heating and storage on the stability and discoloration of anthocyanins

The heating effect at 70, 80, 90 and 99.5 °C for 20, 40, 60, 80 and 100 minutes and storage at 30, 40, 50, 60 and 70 °C for 7, 14, 21, and 28 days all showed good correlations to anthocyanin content and color parameters L^* , a^* , b^* , C^* , H° and ΔE . Figures 1a and 2a showed the effect of temperature,

duration of heating and storage on decreasing anthocyanin levels especially on storage, where higher heating temperatures and longer storage led to decreased anthocyanin levels. The higher temperatures (70 to 99.5 °C) with shorter times (20 to 100 minutes) showed a decrease in anthocyanin levels and smaller color changes compared to storage at lower temperatures (30 to 70°C) with longer time (7 to 28 days).

Higher temperatures and longer storage caused the lighter color (lightness or L^*), the degree of hue (H^o) and ΔE to be greater, but vice versa to chroma (C^*), redness (a^*) and yellowness (b^*) parameters that decreased at elevated temperatures (Figure 1b-g and 2d-g). As the previous researchers [25] pointed out, the increase in L^* values was caused by the formation of lightness or translucent extracts so that the color faded and the yellow chalcone species were formed, while the changes in H^o , would be associated with the formation of yellow chalcone species (H^o towards 90°). The decrease in a^* and C^* values was associated with degradation of anthocyanins monomer or color saturation [8, 25].

The general consensus was that anthocyanin pigments were readily destroyed by heat during the processing and storage of foods [8], the author also stated that the degradation was unaffected by O_2 , but it was greatly accelerated by heat. The ring-opening and degradation of anthocyanins were found to be the main factors that were responsible for the color change at high temperature [26]. Many studies indicated that the anthocyanin concentrations and color stability decreased at all temperatures and more rapidly at higher temperatures as affected by heat treatment and during storage [25, 27-31].

The heating treatment caused the equilibrium to shift towards the chalcone, however, the reversion of the chalcone was slow. The flavonoid structure was open to form a chalcone (an aromatic ketone and an enone that formed the central core of the variety of important biological compounds) which was further degraded to form brown products [32].

Increasing the temperature showed the k -value of anthocyanin degradation and the color parameters L^* , a^* , b^* , C^* , H^o and ΔE at 99.5°C were 4.67, 2.25, 2.18, 2.47, 2.21, 3.63, and 1.18 times respectively higher than those at 70°C, while increasing the temperature during storage shows the k -value of anthocyanin degradation and the color parameters L^* , a^* , b^* , C^* , H^o and ΔE at 70°C were 13.8, 4.2, 16.0, 1.9, 7.1, 9.8, and 1.3 times respectively higher than those at 4°C. Anthocyanin damage and color fading ratio in low-temperature storage were much higher than heating at high temperatures with a shorter time. It could be stated that anthocyanins were more unstable on heating with lower temperatures (30 to 70°C) but with a longer time (7 to 28 days) than that at higher temperatures (70 to 99.5°C) with a very short time (<100 minutes). Thus, to avoid damage, it was better to produce the anthocyanin immediately to be processed than storage.

3.2 Thermal degradation kinetics of anthocyanin and color changes due to heat treatment

Kinetics of anthocyanin degradation and a^* , C^* , ΔE color parameters in heating treatments followed a first-order reaction as illustrated in equation (7). In previous studies, anthocyanin heating degradation kinetics followed a first-order reaction [23, 27-31, 33-36] a^* and C^* color parameters and the ΔE also followed first-orders [25]. They also said that the temperature-dependence degradation rate constant was represented by the Arrhenius equation.

Table 1 showed the parameters of anthocyanin degradation kinetics and L^* , a^* , b^* , C^* , H^o colors and ΔE value from the roselle petal extract. It was seen that there was a^* and C^* decrease in anthocyanin levels along with the increase in temperature and heating time, from 70 to 99.5 °C for 20, 40, 60, 80 and 100 minutes, as well as a^* , b^* and C^* color parameter, while L^* , H^o color, and ΔE increased with longer time. The decreased values of a^* and C^* would be related to degradation of monomeric anthocyanin, and increased value of L^* would be related to the formation of translucent extracts due to the color fading, while changes of b^* and H^o value would be associated to the formation of yellow chalcone species (H^o towards 90 °C) [25]. Increased k -value during heating (rate of damage) of anthocyanin (0.0006 to 0.0028), L^* (0.0004 to 0.0009), a^* (0.0011 to 0.0024), b^* (0.0019 to 0.0047), C^* (0.0014 to 0.0031), H^o (0.0008 to 0.0029) and ΔE (0.0288 to 0.0341) indicated higher levels of anthocyanin damage and decreased color intensity so that the roselle extract appeared to fade due to reduced red color as decreased levels of anthocyanin.

To determine the effect of temperature on the parameter studied, the constant obtained from equation (6) and (7) were fitted to an Arrhenius type equation (Equation (10)). The calculated anthocyanin activation energy (E_a) was 53.69 KJ/ mol. $^oK^{-1}$, while L^* , a^* , b^* , C^* , H^o and ΔE activation energy were 29.82, 29.43, 32.35, 29.60, 45.86 and 5.92 KJ /mol. $^oK^{-1}$ respectively, both were heated at temperatures 70, 80, 90 and 99.5 °C (Table 1). The lower E_a can be associated with the increased temperature dependence of the anthocyanins degradation rate [33]. Anthocyanins had considerably lower k -values, although, with a higher E_a , these anthocyanins were less stable to thermal degradation compared to the other color parameters (L^* , a^* , b^* , C^* , and H^o). However, as shown in Table 1, ΔE had a k -value and E_a lower than anthocyanin. [31] has done research on blood orange juice which stated that anthocyanins degradation increased with increasing heating temperature and time wherein the comparison of k -values at selected temperatures showed the following order: $k_{90^oC} > k_{80^oC} > k_{70^oC}$. Furthermore, the results suggested that the decrease of visual color was fastest at 90°C while lowest at 70°C, which agreed with that of anthocyanins.

3.3 Thermal degradation kinetics of anthocyanin and color changes due to during storage

The thermal degradation profile of anthocyanin and color parameter (L^* , a^* , b^* , C^* , H^o and ΔE) during storage at 4, 30, 40, 50, 60 and 70 °C were shown in Table 2. Based on Table 2, thermal degradation of the anthocyanin, a^* , C^*

color and ΔE value were corresponding to the first order kinetics. The temperature dependence of thermal degradation values (k -values) was determined from Arrhenius equation. Increased k -value during storage (rate of damage) of anthocyanin was 0.0096 to 0.1326, L^* 0.0052 to 0.0217, a^* 0.0046 to 0.0733, b^* 0.0086 to 0.0164, C^* 0.0060 to 0.0426, H^o 0.0040 to 0.0392 and ΔE 0.1031 to 0.1357. The k -value of anthocyanin and color indicated that the increase in temperature caused an increase in the k -value, thus the storage temperature had a strong effect on the degradation rate of anthocyanin and color changes. Based on a similar study[24, 37] mentioned that storage temperature affected on the degradation of anthocyanins, the results clearly indicated that anthocyanin degradation increased with increasing storage temperature and time.

Increased k -value during storage (rate of damage) of anthocyanin indicated higher levels of anthocyanin damage and decreased color intensity so that the roselle extract appeared to fade due to a reduced red color as the decreased levels of anthocyanin. The half-life ($t_{1/2}$) values of anthocyanin and L^* , a^* , b^* color parameters during storage were reported that lower temperature contributed to a higher half-life compared to a higher temperature (Table 2). In this study, the lowest value of the E_a was obtained for ΔE value (3.12 KJ/mol. $^{\circ}K^{-1}$) during storage, while the highest was in the anthocyanin (32.00 KJ/mol. $^{\circ}K^{-1}$).

Temperature is another parameter affecting the color stability of anthocyanin. When the temperature increases, the stability of anthocyanin decreases, hence a^* color change can be observed[34]. Table 2 showed anthocyanin degradation and color changes in rosella petal extract during storage on the k , $t_{1/2}$ and E_a value. The Energy activation of anthocyanin and L^* , a^* , b^* , C^* , H^o and ΔE color during storage in Table 2 are 32.00, 17.86, 30.10, 7.25, 21.72, 23.23 and 3.12 KJ/mol. $^{\circ}K^{-1}$, respectively. Higher E_a signified the greater heat sensitivity during storage at all temperature and time, which meant that the temperature dependence of anthocyanin degradation was also greater.

All the samples had decreased anthocyanin levels and the colors were noticeably faded along with the increase in temperature and storage time. This was due to the instability of anthocyanin in the extract of roselle petals. Anthocyanins degradation increased with increasing heating temperature and time. Due to elevating temperatures, k -value and the time needed for 50% degradation of anthocyanins and colour changes of L^* , a^* , b^* , C^* , H^o and ΔE value during storage at 4 $^{\circ}C$ when compared to storage at 70 $^{\circ}C$, the ratio was equal to the anthocyanin damage (k) value and the color parameter changes were at 70 $^{\circ}C$ to 4 $^{\circ}C$. The comparison of k -values at selected temperatures showed the following order: $k_{70^{\circ}C} > k_{60^{\circ}C} > k_{50^{\circ}C} > k_{40^{\circ}C} > k_{30^{\circ}C} > k_{4^{\circ}C}$. It was clearly seen that anthocyanin damage and color fading were strongly affected by higher storage temperatures.

4. Conclusions

The results from the present study provided detail information about the potential use of rosella as a source of natural colorants for the food industry. The increasing temperature during heating and storage increased

the degradation rate of anthocyanin content followed first-order reaction kinetics. The color stability of rosella petal extract anthocyanins in heat treatment and during storage showed that the changes in Hunter color values were according to zero-order kinetics for L^* , b^* , and H^o parameters, while for a^* , C^* , and ΔE parameters according to first-order degradations kinetics. The degradation of anthocyanins showed a positive correlation with a^* , b^* and C^* value, while it showed a negative correlation with L^* , H^o and ΔE . It is clear that anthocyanin is more stable at higher temperatures with a shorter time than at lower temperatures for longer periods. To avoid damage, thus, the anthocyanin should be immediately used and processed.

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Table

1: The effect of temperature on the k-value of anthocyanin degradation and color changes in rosella petal extract

Index	Temperature (°C)	Kinetic equation	k-value (min ⁻¹)	Ea (KJ/ mol.°K ⁻¹)
Anthocyanin	70	-0.0006x - 0.0024	0.0006 (0.9563) ^a	53.69
	80	-0.0012x + 0.0007	0.0012 (0.9742)	(0.9868) ^a
	90	-0.0017x - 0.0089	0.0017 (0.9561)	

	99.5	-0.0028x - 0.0090	0.0028 (0.9838)	
L^*	70	0.0004x + 0.9982	0.0004 (0.9274)	29.82
	80	0.0005x + 0.9985	0.0005 (0.9620)	(0.9921)
	90	0.0007x + 1.0004	0.0007 (0.9309)	
a^*	99.5	0.0009x + 1.0040	0.0009 (0.9449)	
	70	-0.0011x + 0.0022	0.0011 (0.9585)	29.43
	80	-0.0012x - 0.0013	0.0012 (0.9356)	(0.9396)
b^*	90	-0.0018x + 0.0140	0.0018 (0.9547)	
	99.5	-0.0024x + 0.0055	0.0024 (0.9609)	
	70	-0.0019x + 1.000	0.0019 (0.9766)	32.35
C^*	80	-0.0022x + 1.0039	0.0022 (0.9768)	(0.9361)
	90	-0.0030x + 0.9916	0.0030 (0.9704)	
	99.5	-0.0047x + 1.0030	0.0047 (0.9767)	
H^p	70	-0.0014x + 0.0022	0.0014 (0.9712)	29.60
	80	-0.0015x + 0.0042	0.0015 (0.9648)	(0.9237)
	90	-0.0022x + 0.0091	0.0022 (0.9653)	
ΔE	99.5	-0.0031x + 0.0065	0.0031 (0.9711)	
	70	-0.0008x + 1.0003	0.0008 (0.9211)	45.86
	80	-0.0009x + 1.0038	0.0009 (0.9358)	(0.8870)
ΔE	90	-0.0014x + 0.9889	0.0014 (0.9115)	
	99.5	-0.0029x + 1.0146	0.0029 (0.9392)	
	70	0.0288x + 32.699	0.0288 (0.8750)	5.92
ΔE	80	0.0302x + 32.739	0.0302 (0.8713)	(0.9733)
	90	0.0316x + 32.978	0.0316 (0.8097)	
	99.5	0.0341x + 33.138	0.0341 (0.7789)	

^aCoefficients of determination (R^2) shown in parenthesis

Table 2: Anthocyanin degradation and color changes in rosella petal extract during storage on the k-value, $t_{1/2}$, and E_a value

Index	Temperature ($^{\circ}\text{C}$)	Kinetic equation	k-value (min^{-1}) ^a	$t_{1/2}$ (d)	E_a (KJ/mol $^{\circ}\text{K}$)
Anthocyanin	4	-0.0096x - 0.0366	0.0096 (0.9221) ^a	72	32.00
	30	-0.0301x - 0.1130	0.0301 (0.9220)	23	(0.9931) ^a
	40	-0.0531x - 0.1437	0.0531 (0.9293)	13	
	50	-0.0790x - 0.2146	0.0790 (0.9575)	8	
	60	-0.0975x - 0.3192	0.0975 (0.9398)	7	
	70	-0.1326x - 0.4925	0.1326 (0.9253)	5	
	L^*	4	0.0052x + 0.9833	0.0052 (0.9156)	96
30		0.0079x + 0.9717	0.0079 (0.9029)	63	(0.9653)
40		0.0103x + 0.9974	0.0103 (0.9824)	49	
50		0.0142x + 1.0489	0.0142 (0.9224)	35	
60		0.0188x + 1.0766	0.0188 (0.9147)	27	
70		0.0217x + 1.0728	0.0217 (0.9232)	23	
a^*		4	-0.0046x + 0.0034	0.0046 (0.9246)	151
	30	-0.0070x - 0.0115	0.0070 (0.9516)	99	(0.7950)
	40	-0.0094x - 0.0137	0.0094 (0.9670)	74	
	50	-0.0344x + 0.0655	0.0344 (0.9726)	20	
	60	-0.0531x + 0.1015	0.0531 (0.9738)	13	
	70	-0.0733x - 0.0651	0.0733 (0.9851)	9	
	b^*	4	-0.0086x + 1.0150	0.0086 (0.9424)	58
30		-0.0143x + 1.0240	0.0143 (0.9675)	35	(0.8613)
40		-0.0144x + 0.9991	0.0144 (0.9724)	35	
50		-0.0145x + 0.9883	0.0145 (0.9710)	34	
60		-0.0155x + 0.9382	0.0155 (0.9000)	32	
70		-0.0164x + 0.9141	0.0164 (0.8629)	30	
C^*		4	-0.006x + 0.0079	0.0060 (0.9485)	116
	30	-0.0097x + 0.0015	0.0097 (0.9857)	71	(0.8511)
	40	-0.0117x - 0.0071	0.0117 (0.9861)	59	
	50	-0.0283x + 0.0397	0.0283 (0.9827)	24	
	60	-0.0379x + 0.0255	0.0379 (0.9935)	18	
	70	-0.0426x - 0.1226	0.0426 (0.9950)	16	
	H^p	4	-0.0040x + 1.0130	0.0040 (0.8144)	125
30		-0.0082x + 1.0390	0.0082 (0.8349)	61	(0.8482)
40		-0.0067x + 1.0205	0.0067 (0.8174)	75	
50		0.0129x + 0.9433	0.0129 (0.8628)	39	
60		0.0266x + 0.8703	0.0266 (0.8464)	19	
70		0.0392x + 0.9985	0.0392 (0.9578)	13	
ΔE		4	0.1031x + 32.216	0.1031 (0.9470)	6.7
	30	0.1126x + 32.536	0.1126 (0.8721)	6.2	(0.8726)

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	40	0.1147x + 32.755	0.1147 (0.7939)	6.0	
	50	0.1301x + 33.027	0.1301 (0.7382)	5.3	
	60	0.1362x + 33.165	0.1362 (0.7073)	5.1	
	70	0.1357x + 33.399	0.1357 (0.6293)	5.1	

^aCoefficients of determination (R^2) shown in parenthesis

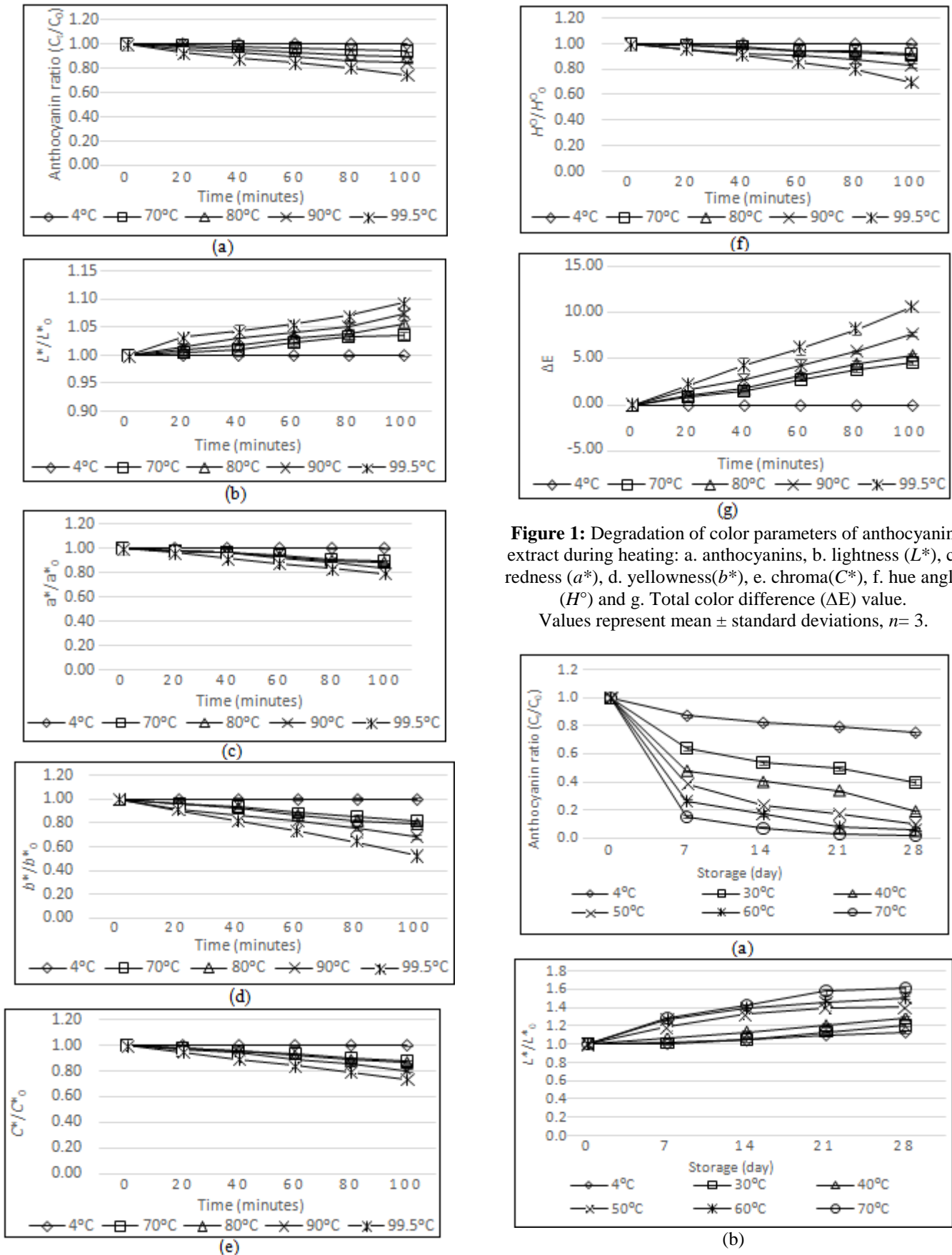
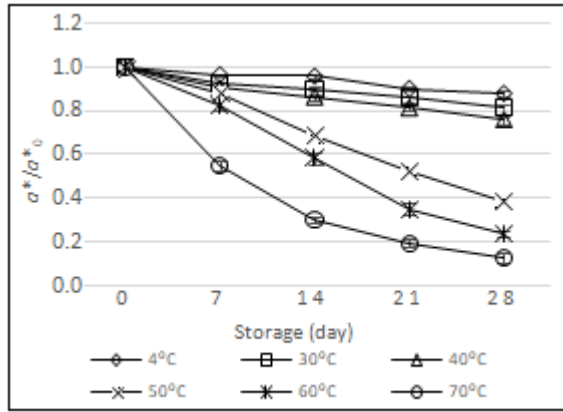
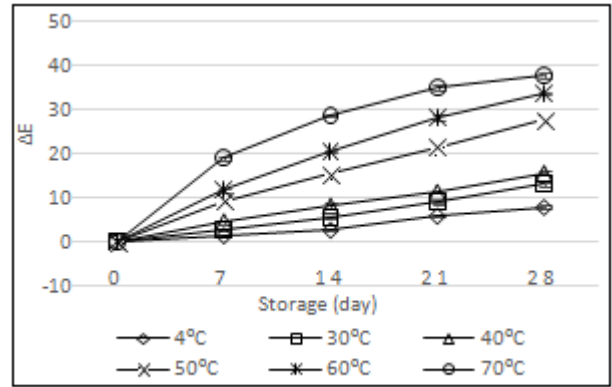


Figure 1: Degradation of color parameters of anthocyanin extract during heating: a. anthocyanins, b. lightness (L^*), c. redness (a^*), d. yellowness(b^*), e. chroma(C^*), f. hue angle (H°) and g. Total color difference (ΔE) value. Values represent mean \pm standard deviations, $n= 3$.



(c)

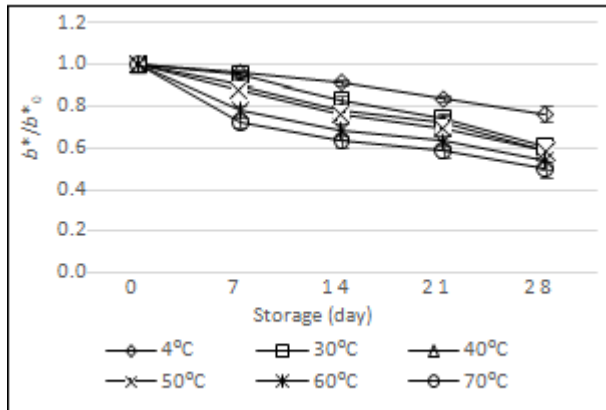


(g)

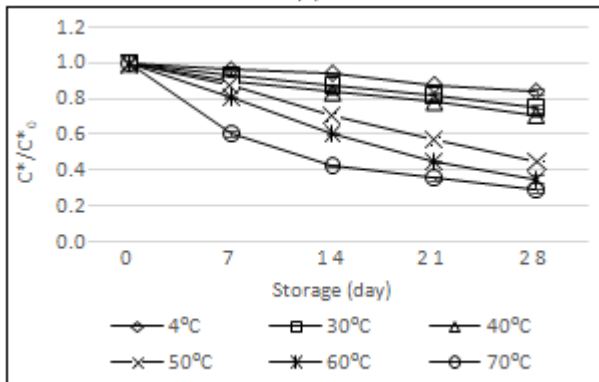
Figure 2: Degradation of color parameters of anthocyanin extract during storage

A. anthocyanins, B. lightness (L^*), C. redness (a^*), D. yellowness (b^*), E. chroma (C^*), F. hue angle (H°) and G. Total color difference (ΔE) value.

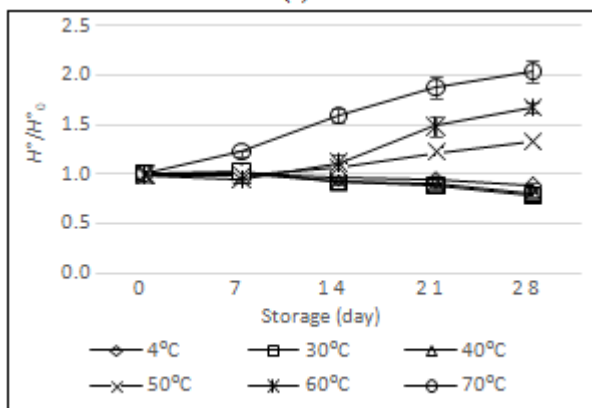
Values represent mean \pm standard deviations, $n=3$.



(d)



(e)



(f)