Improvement of Anthocyanin Stability in Butterfly Pea Flower Extract by Co-pigmentation with Catechin

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Abstract. Most of the food processing operations involve the use of heat which generally causes alteration, and degradation of natural pigments, resulting in lower stability. One of the stability enhancement methods is co-pigmentation. This study aimed to determine effect of catechin co-pigment on stability of anthocyanins in *Clitoria ternatea* (or butterfly pea flower) extract. Degradation kinetics of anthocyanins in the extract were evaluated at three temperatures (28, 60, and 90°C). The effect of co-pigment ratio (catechin: anthocyanins at 1:1, 50:1 and 100:1 by weight) on the stability of anthocyanin extract at 90°C was determined by the pH differential method. It was found that anthocyanin degradation followed the zero- order kinetics at all temperatures; the degradation rate increased as the temperature increased. At a lower pH, anthocyanins became more stable. An increase in the co-pigment ratio significantly retarded the degradation anthocyanins at 90°C. In addition, co-pigmentation also intensified the color of butterfly pea extract. The highest anthocyanin stability was obtained at co-pigment ratio of 100:1.

1 Introduction

Anthocyanins are bioactive compounds available in a large variety of fruits, vegetables, flowers and other plant tissues, such as, berries, cabbage, blood orange, grape and butterfly pea. The anthocyanins are polyphenols having antioxidant activities, which are responsible for some biological activities and health-promoting properties in preventing or lowering the risk of cardiovascular disease, diabetes, arthritis and cancer [1]. Besides being used as an antioxidant to fortify in food products, anthocyanins are also a predominant choice for natural food colorants providing the bright red-orange to blue-violet. Color is one the most important characteristics of food products to bring a good impression for consumer acceptance. Anthocyanins are the largest and the most important group of water-soluble pigments in nature with comparatively low toxicity [1, 2].

Clitoria ternatea (butterfly pea) is one of the herbs that are rich in anthocyanins in its petals. It is commonly used as a colorant in Thai beverages and traditional desserts. In addition, many food industries have shifting their interest towards using anthocyanins from natural sources as a food colorant instead of the artificial one. However, the natural food colorants often have stability problem. The stability of anthocyanins depends on a combination of various factors, such as structure and concentration of the anthocyanins, pH, temperature and the presence of complexing agents (i.e., phenols and metals) [3, 4].

Thermal processing used in the food industry can cause anthocyanin degradation. Many researchers have proposed various pretreatments to increase extraction yield and to enhance the stability of such color pigments. Chemical pretreatments have been proposed to enhance the stability of pigments by being associated with co-pigments, such as flavonoids, alkaloids, amino acids, organic acids, metals, phenolics and anthocyanins themselves. The role of such co-pigments is to protect anthocyanins (i.e. flavylium ion) from the nucleophilic addition of water; otherwise, the flavalium ion becomes pseudobase or colorless [3, 5].

The purpose of this research was to determine effect of co-pigment ratio on stability of anthocyanins in butterfly pea extract. The kinetics of anthocyanin degradation was studied at different temperatures and the stability of anthocyanins was evaluated at 90°C. Catechin, which is a natural compound often found in tea, cocoa, berries and red wine, was used in co-pigmentation study because it contains high antioxidant activity and high phenolic compounds.

2 Materials and methods

2.1 Material

Dried butterfly pea flower (*Clitoria ternatea*) was purchased from a local supermarket in Bangkok, Thailand and stored in the dark at room temperature.

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2.2 Preparation of butterfly pea extract

The butterfly pea extract was prepared just enough for each experiment. Butterfly pea powder was prepared by grinding the dry butterfly pea flower at 25,000 rpm for 10 s using a grinding machine (Mill Powder Tech, RT-N04, Taiwan) and then sieved through 30-mesh screen. The dried butterfly pea powder was mixed with water at 1:10 ratio and stirred at 900 rpm for 5 min using a magnetic stirrer (Thermo Scientific, SP131825, China). The mixture was centrifuged at 10,000 rpm for 10 min (Hitachi, Himac CF15R, Japan) and filtered through no.1 filter paper to obtain a butterfly pea extract. The extract was kept in a glass vial covered with aluminium foils until use.

2.3. Determination of anthocyanin degradation kinetics at different temperatures

Kinetics of anthocyanin degradation were determined at 28°C (room temperature), 60°C and 90°C for 60 min. A plastic tube filled with 5-mL sample was capped and covered with aluminium foil before placing in a water bath (Hipoint Kaohsiung, SB-7D, Taiwan) at the desired temperature. After heating for 0 (control), 5, 10, 20, 40 and 60 min, the sample was rapidly cooled in iced water for 5 min to stop the reaction. Total anthocyanin content was determined by pH-differential method [6]. Stability of anthocyanins was reported in terms of degradation rate and half-life. Zero, first and second order kinetic models were explored and the model that best fit the results was selected based on the highest R^2 and the lowest RMSE values.

2.4 Determination of effect of co-pigment ratio on anthocyanin stability

The pH of butterfly pea extract was adjusted to 3.5 by citric acid (Chang Shou Chemical, Co. Ltd., China). Catechin (Chen Yu Biological Technology, Co. Ltd., Taiwan) was added into the butterfly pea extract at the co-pigment to anthocyanins ratio of 1:1, 50:1 and 100:1. The sample was then mixed by vortex mixer and agitated in a water bath shaker at 25±1°C for 30 min. The thermal stability of co-pigment complexation was determined at 90°C. After heating for 0 (control), 5, 10, 20, 40 and 60 min, the sample was rapidly cooled in ice water for 5 min. Total anthocyanin content was determined by pH-differential method [6]. Stability of anthocyanins was reported in terms of degradation rate and half-life.

Change in absorption spectra of the extracts was also monitored by a microplate spectrophotometer using the wavelength between 400-700 nm (visible range). Shifting in absorption intensity and wavelength of the spectra are indicators of co-pigmentation level (or number of conjugated structure) [7].

2.5 Statistical Analysis

Statistical analysis was conducted using IBM SPSS Statistics Version 19.0 (IBM SPPS Inc., USA). The experiments were carried out in triplicate. Data were expressed as mean ± standard deviation. Analysis of Variance (ANOVA) was used to analyze effect of treatments at $\alpha = 0.05$ and Duncan's multiple range test was used to analyze the differences among treatments.

3 Results and discussion

3.1 Kinetics of anthocyanin degradation at different temperatures

Degradation profiles of anthocyanins at different temperatures were illustrated in Fig. 1. It was found that anthocyanin content decreased as the heating time increased. In addition, at the same period, the higher the temperature, the lower the anthocyanin content.

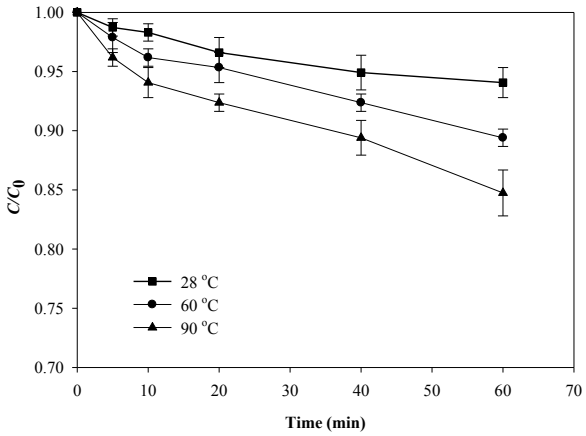

Fig. 1. Degradation of anthocyanins at different temperatures.

The experimental results were fit with zero, first and second order models as shown in Table 1. It was found that the degradation kinetics of anthocyanins extracted from butterfly pea flower followed the zero-order reaction which is contradicted to the first-order reaction reported by Wang and Xu [8]. The anthocyanin content at a given time and its half-life was determined by the following equations.

$$C = -kt + C_0 \quad (1)$$

$$t_{1/2} = C_0/2k \quad (2)$$

where C_0 = an initial anthocyanin content (mg/L)
C_t = an anthocyanin content after time t (min) of heating (mg/L)
k = the reaction constant or the rate of anthocyanin degradation (min^{-1})
$t_{1/2}$ = half-life of anthocyanins (min).

The half-life ($t_{1/2}$) of anthocyanins at 28, 60 and 90°C are shown in Table 2. Significant increase in the degradation rate (k) was observed when the temperature increased. The value of $t_{1/2}$ significantly decreased when the temperature increased from 28 to 60 and 90°C ($P < 0.05$). Although the results disagreed with the previous studies, which indicated that the thermal degradation of anthocyanins in butterfly pea petal extract followed the

first-order kinetics [9, 10], half-life values of anthocyanins in butterfly pea extract at high temperatures (Table 2) were comparable to 5.25 h at 70°C reported in the previous study [10].

Table 1. Kinetic models of anthocyanin degradation at different temperatures.

T (°C)	Equation	R^2	RMSE
	Zero-order Model: $C = -kt + C_0$		
28	y = -0.1219x+125.28	0.9379	0.6636
60	y = -0.2039x+124.72	0.9645	0.8285
90	y = -0.2805x+123.41	0.9388	1.5162
	First-order Model: $\ln(C/C_0) = -kt$		
28	y = -0.0012x	0.8844	1.4930
60	y = -0.0020x	0.9257	1.2244
90	y = -0.0029x	0.8744	2.2608
	Second-order Model: $(1/C) - (1/C_0) = kt$		
28	y = 0.00002x	0.8981	0.9016
60	y = 0.00002x	0.9376	18.6905
90	y = 0.000009x	0.8921	8.1894

Table 2. Half-life of anthocyanins at different temperatures.

T (°C)	$t_{1/2}$ (h)
28	8.63
60	5.16
90	3.75

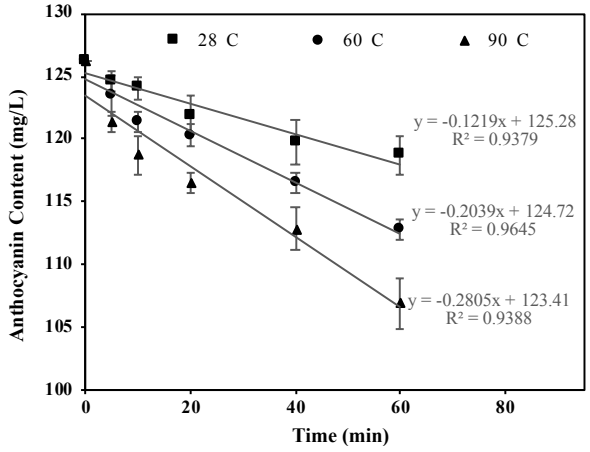

Fig. 2. Zero order kinetics of anthocyanin degradation at different temperatures.

3.2 Effect of co-pigment ratio on anthocyanin stability

Fig. 3 showed degradation of anthocyanins at 90°C using different co-pigment ratios. For all co-pigment ratios, the anthocyanin content decreased as the heating time increased. An increase in co-pigment ratio could slow down the rate of anthocyanin degradation.

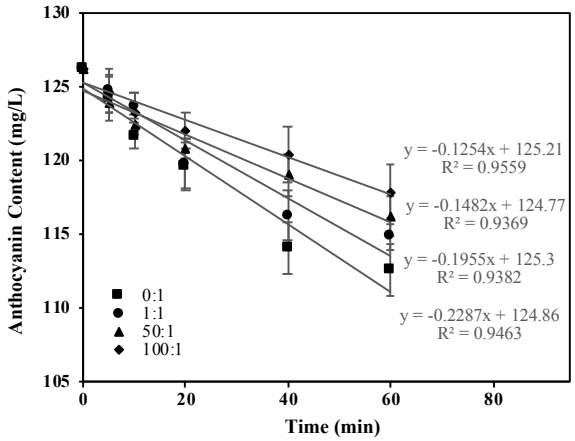

Fig. 3. Degradation of anthocyanins at 90°C using different co- pigment ratios.

Similar to section 3.1, the results were fit with zero, first and second order models as shown in Table 3. The model that gave the highest R^2 and lowest RMSE was zero-order model. Thus, the degradation of anthocyanin complex followed linear model. The half-life ($t_{1/2}$) values of anthocyanins at all co-pigment ratios are presented in Table 4. The rate constant (k) decreased almost twice when the co-pigment ratio increased to 100:1 which corresponded to an increase in the half-life from 4.60 to 8.39 h. A significant increase in half-life of anthocyanins could be obtained by adding catechin at 50:1 ratio ($P < 0.05$). Similar result was also reported in the previous work [11], which indicated that the addition of catechin and other phenolic compounds reduced reaction rate constants of anthocyanin reduction in red current juice and increased its half-life.

Table 3. Kinetic models of anthocyanin degradation at 90°C using different co- pigment ratios.

Ratio	Equation	R^2	RMSE
	Zero-order Model: $C = -kt + C_0$		
0:1	y = -0.2287x+124.86	0.9463	1.1525
1:1	y = -0.1955x+125.30	0.9382	1.0624
50:1	y = -0.1482x+124.77	0.9369	0.8139
100:1	y = -0.1254x+125.21	0.9559	0.5700
	First-order Model: $\ln(C/C_0) = -kt$		
0:1	y = -0.0022x	0.9232	17.1582
1:1	y = -0.0018x	0.9246	14.0829
50:1	y = -0.0015x	0.8611	11.7493
100:1	y = -0.0012x	0.9038	9.4464
	Second-order Model: $(1/C) - (1/C_0) = kt$		
0:1	y = 0.00002x	0.9328	1.3911
1:1	y = 0.00001x	0.9311	2.3652
50:1	y = 0.00001x	0.8744	1.5557
100:1	y = 0.00001x	0.9124	0.8196

Table 4. Half-life of anthocyanin complex at 90°C using different co-pigment ratios.

Ratio	$t_{1/2}$ (h)
0:1	4.60
1:1	5.38
50:1	7.10
100:1	8.39

An increase in stability of anthocyanin after co-pigmentation could be explained by an alteration of its structure. The structure of catechin composes of two benzene rings (called the A- and B-rings) and a dihydropyran heterocycle (the C-ring) with a hydroxyl group on carbon 3 [12]. Catechin has a similar structure to anthocyanin molecule and it is the building blocks of pro-anthocyanidins. The formation of co-pigment (Fig. 4) could be described by intermolecular co-pigmentation effect [13]. Co-pigmented anthocyanin complex tended to be more stable than the native form of anthocyanin during processing and storage either in the form of interlock or parallel complex [14]. Anthocyanin structure is a planar and has an ability to produce delocalized π-electrons or in π stacking.

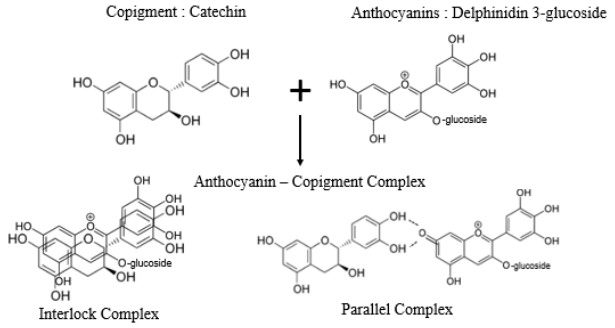

Fig. 4. Intermolecular interaction created complex formations between delphinidin-3-glucoside and catechin. (Modified from [15])

Fig. 5 presented the absorption spectra of butterfly pea extract at different co-pigment ratios. There was a significant shift in amplitude when higher content of catechin was added, but no shift in maximum wavelength was observed. Thus, the magnitude of amplitude shifts directly proportional to the co-pigment ratio. The higher absorbance intensity indicated that more conjugated structures were form and the stability of anthocyanins was extended [7]. These results implied that stability of anthocyanins increased with an increasing amount of catechin (or co-pigment ratio). In addition, the color intensity was also increased as the co-pigment ratio increased. The highest stability could be obtained at the co-pigment ratio of 100:1.

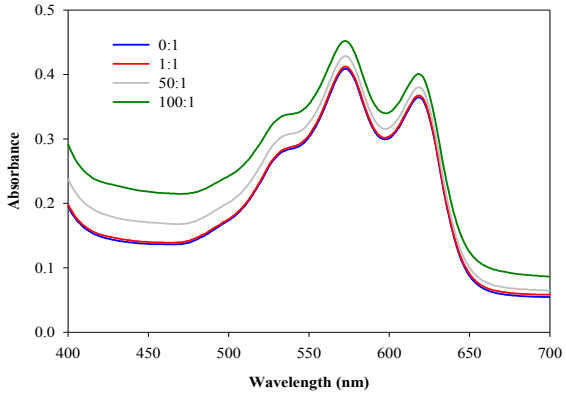

Fig. 5. Absorbance of butterfly pea extract at different co-pigment ratios.

4 Conclusion

The degradation kinetics of the anthocyanin extract obtained from dried butterfly pea flower followed the zero-order reaction or linear model. The half-life values of anthocyanins at 28, 60 and 90°C were 8.63, 5.16 and 3.75 h, respectively. An addition of catechin to form co-pigment could enhance the stability of the anthocyanins. At 90°C, anthocyanins in butterfly pea extract had the highest stability at the co-pigment ratio of 100:1. In addition, the co-pigmentation intensified the blue color of the extract indicated by increasing absorbance intensity. This finding could be applied to increase the stability of natural colorant from butterfly pea extract in food and beverage industries where heating is involved.

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