Effects of pH and anthocyanin concentration on color and antioxidant activity of *Clitoria ternatea* extract

Kuntapas Kungsuwan, Kanjana Singh, Somchai Phetkao and Niramon Utama-ang*

Abstract

Clitoria ternatea flower is commonly used in Thailand as a source of natural coloring for food and cosmetics. Its color pigments are mostly comprised of anthocyanins which show high antioxidant activity and ability to change color according to pH. These benefits can be applied to produce innovative products such as antioxidant beverages and intelligent films which can sense pH change. Thus, to evaluate possibility of the applications, changes in color, antioxidant activity and color acceptance of crude anthocyanin extracts had been evaluated over a pH range of 3 to 9 and different anthocyanin concentration from 10 to 20 mg/100 mL of the extract using response surface methodology. As a result, pH was a major factor influencing both color and antioxidant activity, while anthocyanin concentration showed only a minor influence. Although color acceptance was not significant, the change in color of extracts was apparent; for instance, the color of the extract was red in acidic solution, blue in neutral solution and green in basic solution. Furthermore, the antioxidant activity showed high activity at acidic pH and gradually decreased as pH increased to basic value. Consequently, the optimum point yielding highest antioxidant activity in this study was at pH 3 and 20 mg/100 mL anthocyanin concentration.

Keywords: *Clitoria ternatea*, anthocyanins, color, antioxidant activity and pH

1. Introduction

Clitoria ternatea, called Butterfly Pea, is tendrils grown in Thailand. There are many common names including un-chan, uang-chan and dang-chan. Characteristics of the butterfly pea are a perennial herbaceous plant with elliptic and obtuse leaves. It grows as a vine in moist and neutral soil. The important feature about this plant is its vivid deep blue flowers; solitary with light yellow markings (Singh and Tiwari, 2012). In Thailand, butterfly pea flower is widely used as a natural food coloring.

Division of Product Development Technology, Faculty of Agro-Industry, Chiang Mai University, Chiang Mai 50100 Thailand

* Corresponding author, e-mail: niramon.u@cmu.ac.th

A Blue color of butterfly pea flowers are usually used in Thai dessert such as Khanom Chan and Khanom Ralai. In fact, the pigments of butterfly pea petals are anthocyanins which are water soluble and stable in mild acid solutions (Li et al., 2013). In addition, the color of the anthocyanins can range from deep blue to magenta depending on the pH of a surrounding (Wangsintaweekul, 2006). Anthocyanins are color pigments that provide red, violet and blue colors. They are found in many agricultural products including Butterfly Pea flower. Most naturally occurring anthocyanins occur as a glycoside containing one of several aglycone cores. A major aglycone core of Butterfly Pea petals had been identified as kaempferol (Kazuma et al., 2003). This aglycone core can exist as a positively charged oxonium ion in acidic solution, which is called flavylium cation. The ability of anthocyanin to change color could be explained according to Fig 1. In an acidic solution, the oxonium ion structure results in an extended conjugation of double bonds through three rings of the aglycone moiety; as a result, its structure absorbs photons in higher wavelength of visible spectra such as red and magenta. However, addition of base disrupts the conjugation of double bonds and results in absorption of photons in a higher wavelength such as yellow, blue and purple depending on the degree of disruption in the conjugated system. The change of color by increasing the number of conjugated double bonds in the molecule occurs by lowers the energy level of the electronic transition between the ground state and the excited states which results in an emission of photons at a higher wavelength (Chen and Gu, 2013; Chigurupati et al., 2002). Anthocyanins are considered as antioxidants (Yang and Zhai, 2010). Their benefits include a risk reduction of cancers, arthritises, heart diseases and strokes (Hou et al., 2013; Vareed et al., 2006; Zhou et al., 2012; Zhu et al., 2013). Stability of the anthocyanins depends on their structure, concentration of pigments, acidity, pH, light and temperature (Mazza and Miniati, 1993). Kirca et al. (2007) showed that anthocyanins when heated at high temperatures were disintegrated. Furthermore, Torskangerpoll and Andersen (2005) had showed that structure of the anthocyanins extracted from Butterfly pea flower was affected by pH; thus, its stability changed. For instance, acylated anthocyanins showed better stability than nonacylated anthocyanins.

As mentioned earlier, anthocyanins have high antioxidant activity, many health benefits and ability to change color according to pH of the surrounding. Those key advantages can be used to produce functionalized products including antioxidant drinks and smart packaging which can sense pH change and have antioxidant activity. But, to achieve the applications, there are many factors that need to be investigated such as how the color of the extract changes in different pH, what condition yields the highest antioxidant activity and which color consumers prefer for

their health beverages from Butterfly pea flower. Therefore, this research aims to study the effect of pH and anthocyanin concentration on color, consumer acceptance and antioxidant activity of the *Clitoria ternatea* extracts to elucidate on feasibility of the flower extract for their possible applications.

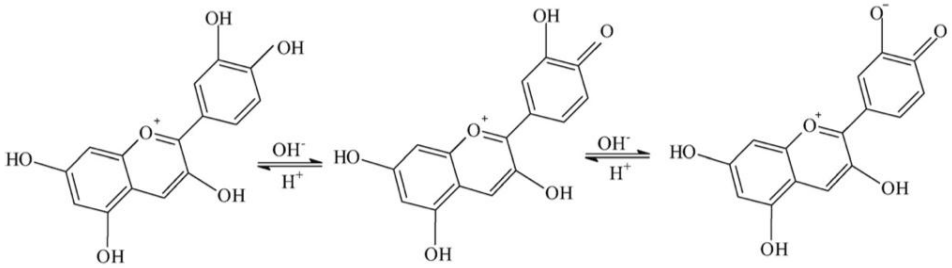

Figure 1. Structural changes of flavylium cation from acidic to basic solution

Source: Chen and Gu (2013)

2. Materials and Methods

2.1 Plant material

The fresh Butterfly Pea Flowers (*Clitoria ternatea*) were bought from Chiang Mai main market (Chiang Mai, Thailand). The flowers were fleshly harvested from the northern area of Chiang Mai province of Thailand in September 2013. The individual flower, without defects and diseases, were selected and de-stemmed. All samples were stored at 4°C standard refrigerator prior to further experiment, which were not more than 3 days after harvested.

2.2 Extraction of anthocyanins from *Clitoria ternatea* petals

An aqueous extraction method was performed by using the different amount of the petals; 10, 20, 30 and 40 g per 100 mL of distilled water. The process started from submerging the petals in the water for 30 min. Then, both petals and water were put in the blender for 30 sec. The resulting mixture was filters through 2 layers of cheesecloths resulting in the *Clitoria ternatea* crude extracts. The amount of total anthocyanin content was measured and regression analysis was performed to correlate the amount of raw flowers required to achieve a specific concentration of anthocyanins. This equation would be used to adjust anthocyanin concentration in the next experiment according to the amount of raw material used during the extraction process.

2.3 Experiment of anthocyanin concentration and pH effect

Parameters in this experiment were anthocyanin concentration and pH value. Extracts in different anthocyanin concentration were adjusted to a different pH value using HCl and NaOH according to experimental plan (Table 1). Then, samples were analyzed for color, antioxidant activity and color acceptance. After the analysis the optimum parameters for the extracts were optimized for the best antioxidant activity.

Table 1. Variation of anthocyanin concentration and pH using central composite design with 2 center points.

Treatment	Code	Parameters	
		Anthocyanins Concentration; X_1 (mg/100 mL)	pH; X_2
1	(1)	10	3
2	a	20	3
3	b	10	9
4	ab	20	9
5	$-\alpha a$	7.93	6
6	$+\alpha a$	22.07	6
7	$-\alpha b$	15	1.76
8	$+\alpha b$	15	10.24
9	cp	15	6
10	cp	15	6

2.4 Color measurement

The visual color was evaluated in two modes; transmission (Ttran) and Reflection (Rsin) using a Hunter Colorimeter (Hunter Lab, Color Quest XE, Hunter Associates Lab Inc., Reston VA, USA). The instrument was standardized each time with a black and a white color (L = 91.10, a = 1.12, b = 1.26) tiles. D65 standard illuminant, corresponding to the natural daylight, and 10° standard observer were used in the measurement. The color values were expressed as L* (lightness), a* (redness (+) / greenness (-)) and b* (yellowness (+) / blueness (-)). Color values were measured in the means of triplicate measurements.

2.5 Determination of total anthocyanin content

Determination of the total amount of anthocyanins (TAC) was adapted from reported spectrophotometric method (Sompong et al., 2011). Anthocyanins were extracted with acidified methanol (methanol and 1 M HCl, 85:15, v/v) with a solvent to sample ratio of 1:10. Absorbance was measured after centrifugation (at 3,000 g for 15 min) at 525 nm against a reagent blank. Cyanidin-3-glucoside-chloride (Sigma-Aldrich, St. Louis, MO, USA) was used as standard pigment, and TAC was expressed as mg cyanidin 3-glucoside equivalent per 100 mL extract.

2.6 Analysis of antioxidant activity

The antioxidant activities were determined using 2, 2-diphenyl-1-picrylhydrazyl (DPPH) purchased form Sigma-Aldrich (St. Louis, MO, USA). The method, adapted from Maisuthisakul et al. (2007), started by mixing 0.1 mL of sample with 4.9 mL DPPH solution (DPPH 4.96 mg in Ethanol 100 mL). The mixtures were placed in the Dark for 30 min; The absorbance of each sample was read at 515 nm using a spectrophotometer (UV–vis model 1601, Shimadzu, Kyoto, Japan). Experiments were carried out three times. Data were expressed as percent inhibition of free radicals compared with the standard DPPH solution. The percentage of DPPH radical scavenging activity of each sample was calculated as shown

$$\text{Scavenging activity (\%inhibition)} = [A_0 - A_1] / A_0 \times 100$$

Where A_0 = Absorbance of the control; A_1 = Absorbance of the sample.

2.7 Sensory acceptance testing

Color acceptance was conducted with 50 consumers. They were screened for their previous experience in beverages from *Clitoria ternatea*. Then, consumers were asked to evaluate color likeness of the samples by sight as if the samples were actual beverages from *Clitoria ternatea*. Ninepoints hedonic scale with values associated to the hedonic terms ranging from 1 “disliked extremely” to 9 “liked extremely” with a central hedonic term of “neither liked nor disliked” with a value of 5 were used in the evaluation.

2.8 Statistical analysis

Regression analysis of anthocyanin extraction was calculated using SPSS Statistics for Windows version 14.0 (SPSS Inc., Chicago, USA) to establish an equation representing anthocyanin concentration based on amount of *Clitoria ternatea* petals used during the extraction process.

Study on the effect of pH and anthocyanin concentration was performed using response surface methodology. The experimental design and result analysis was established using the Design-Expert software version 7.1 (Statease Inc., Minneapolis, USA). The applied experimental design was that of Central Composite Design (CCD) as shown in Table 1.

3. Results and Discussion

3.1 Extraction of anthocyanins from *Clitoria ternatea* petals

The relationship between anthocyanin concentration of the extract and the amount of *Clitoria ternatea* petals used in the extraction is shown in Fig 2. The linear relationship between the two parameters was achieved with p-value equal to 0.01 at 95% confident interval.

$$Y = 3.922 + 0.587\ X\ (R^2 = 0.872)$$

Where, Y is the anthocyanin concentration (mg/100 mL) and X is the amount of *Clitoria ternatea* petal (g). The equation was used to predict anthocyanin concentration from the amount of *Clitoria ternatea* petal.

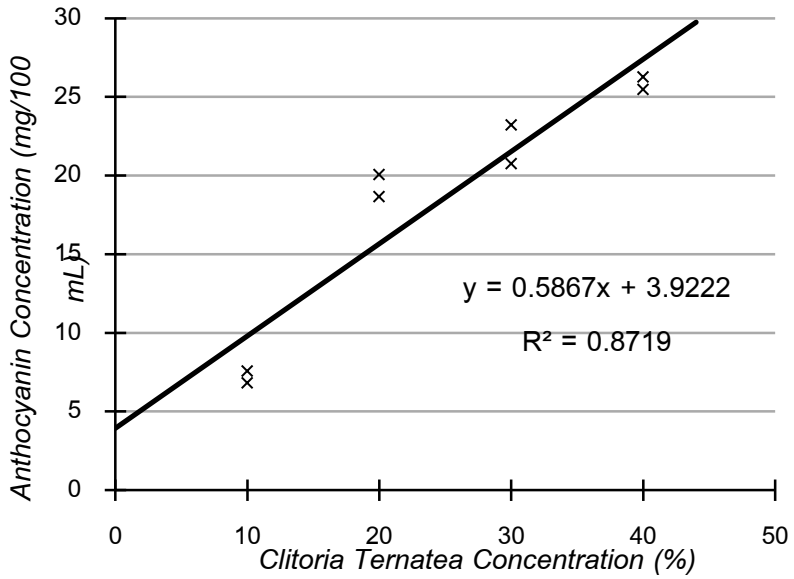

Figure 2. Linear regression of anthocyanin concentration and amount of flower use in the extract

3.2 Anthocyanin concentration and pH effect

Experiment result is shown in Table 2 and 3. Significant parameters were color parameters in transmission mode; L* and a*, color parameters in reflection mode; L*, a* and b*, and antioxidant activity of the extracts. On the other hand, the color acceptance was not significant because color preference between each individual might be different. All mathematical models, p-values and also R^2 of the models are summarized in Table 4. Lack-of-fit testing for all models was not significant with 95% confident interval.

3.2.1 Effect on Color

The color variation in the aqueous solutions of anthocyanin crude extracts was studied in different pH range and anthocyanin concentration. The results are shown in Fig 3a to 3e and Fig 4. The color of the extract in the neutral pH was blue; but when the solution turned more acidic, the color of the crude flower extracts was shifted into the red color region which the bright red-pink color was achieved at pH 1.76. This is shown by a* values of both modes (Fig 3b and 3d) which their values increase as pH decrease. In contrast, when the extracts changed into a basic condition, the color of the extract was shifted to green, a* values decrease as pH increase. The color change of the extract is resulted from a change in structure of anthocyanins which

support more or less conjugated system resulting in the shift of color in visible light spectrum (Walkowiak-Tomczak & Czapski, 2007). Therefore, different color changes between basic and acidic conditions reflect that structural change of anthocyanins in acidic conditions favored conjugated system more than that of basic conditions. Therefore, the color of the extracts in basic condition was shifted to green as indicated by b* value measured in reflection mode (Fig 3e). The small L* values (Fig 3a and 3c) in both modes indicate that the crude extracts were turbid, so the color of the extract were mostly resulted from the reflected light from the samples. Therefore, color measured from the reflection mode is a preferable mode for color measurement in this study.

The coefficients in all color parameter equations (Table 4) suggest that the main factor affecting the color of the extracts were the pH value of the solution. On the other hand, the effect of anthocyanin concentration had shown a little impact on the color of the extract; thus, the result implies that the main reason for color change was the different in anthocyanin structure from pH.

Table 2. Effects of pH and anthocyanin concentration on color parameters.

Treatment	Transmission			Reflection		
	L*	a*	b*	L*	a*	b*
1	0.27 ± 0.08	0.79 ± 0.07	0.16 ± 0.10	26.35 ± 0.01	-0.07 ± 0.04	-0.33 ± 0.06
2	3.44 ± 0.04	23.01 ± 0.06	5.46 ± 0.09	26.75 ± 0.02	1.24 ± 0.02	-1.29 ± 0.02
3	3.80 ± 0.03	24.83 ± 0.11	-11.72 ± 0.11	26.46 ± 0.02	0.96 ± 0.04	-1.61 ± 0.04
4	13.50 ± 0.04	48.00 ± 0.14	-24.63 ± 0.08	26.57 ± 0.01	3.45 ± 0.03	0.04 ± 0.06
5	1.68 ± 0.01	10.45 ± 0.14	-2.85 ± 0.12	26.44 ± 0.02	0.64 ± 0.05	-1.43 ± 0.05
6	0.40 ± 0.05	1.82 ± 0.05	0.40 ± 0.09	26.17 ± 0.01	-0.12 ± 0.05	-0.29 ± 0.05
7	13.46 ± 0.05	18.16 ± 0.04	-36.07 ± 0.05	26.74 ± 0.02	0.92 ± 0.04	-1.82 ± 0.05
8	0.97 ± 0.03	4.15 ± 0.10	0.63 ± 0.09	26.39 ± 0.01	-0.03 ± 0.03	-0.39 ± 0.02
9	23.67 ± 0.01	56.83 ± 0.01	40.38 ± 0.09	27.25 ± 0.01	6.19 ± 0.01	1.16 ± 0.03
10	0.76 ± 0.03	4.82 ± 0.02	1.07 ± 0.23	26.50 ± 0.01	0.62 ± 0.05	-1.67 ± 0.04

Table 3. Effects of pH and anthocyanin concentration on antioxidant activity and color acceptance.

Treatment	DPPH (% Inhibition)	Color Liking
1	16.10 ± 1.92	4.24 ± 1.77
2	57.66 ± 2.08	6.70 ± 1.52
3	36.71 ± 0.89	6.36 ± 1.37
4	32.89 ± 0.78	5.98 ± 1.74
5	32.01 ± 0.92	6.54 ± 1.58
6	4.21 ± 0.66	3.54 ± 1.80
7	15.91 ± 0.31	5.14 ± 2.01
8	8.52 ± 0.01	7.14 ± 1.25
9	41.95 ± 0.31	6.36 ± 2.69
10	56.34 ± 1.00	5.50 ± 2.50

The color change pattern in this study had shown a similarity with the pattern achieved from studies of the color change of anthocyanin extracts form red cabbages which exhibited application as a natural pH indicator and colorant (Chen and Gu, 2013; Chigurupati et al., 2002; Lin et al., 2008; Walkowiak-Tomczak and Czapski, 2007). Likewise, the extract form the *Clitoria ternatea* might be applied as natural pH indicator and colorant for food, cosmetics and pharmaceutical applications; especially, with a smart packaging to monitor pH of food product (Yoshida et al., 2014). For instance, the color parameter a* (Table 4) responsible for the color change from red to green can be used to correlate and predict pH value of the extract. This had proved the applicability of the extract as pH indicator for smart packaging. Hence, the equation of color parameter a* can be a starting framework on how the color on the *Clitoria ternatea* flower extract can be used to monitor pH of a product using the smart packaging.

Table 4. Regression equation of significant parameters in term of standardized factors.

Mathematical Models in Term of Standardized Factors †	**P-value**	**R^2**
L* (Transmission) = 3.80 - 3.59 * X_1 - 6.08 * X_2 + 2.99 * X_2^2	0.0196	0.7860
a* (Transmission) = 17.64 - 5.90 * X_1 - 17.98 * X_2 - 3.43 * X_1^2 + 5.49 * X_2^2 + 5.41 * X_1X_2	0.0065	0.9605
L* (Reflection) = 26.56 - 0.03 * X_1 - 0.26 * X_2	0.0144	0.7021
a* (Reflection) = 0.57 - 0.33 * X_1 - 1.71 * X_2 + 1.02 * X_2^2	0.0063	0.8552
b* (Reflection) = - 1.60 - 0.13 * X_1 + 0.78 * X_2 + 1.05 * X_2^2 + 0.35 * X_1X_2 - 0.65 * X_2^3	0.0082	0.9556
Antioxidant Activity (%Inhibition) = 34.36 + 1.88 * X_1 - 19.62 * X_2 + 0.68 * X_1^2 - 5.84 * X_2^2 - 4.30 * X_1X_2 + 6.21 * X_1^3 + 3.14 * X_2^3	0.0135	0.9961

† X_1 = Anthocyanin Concentration (mg/ 100 mL); X_2 = pH.

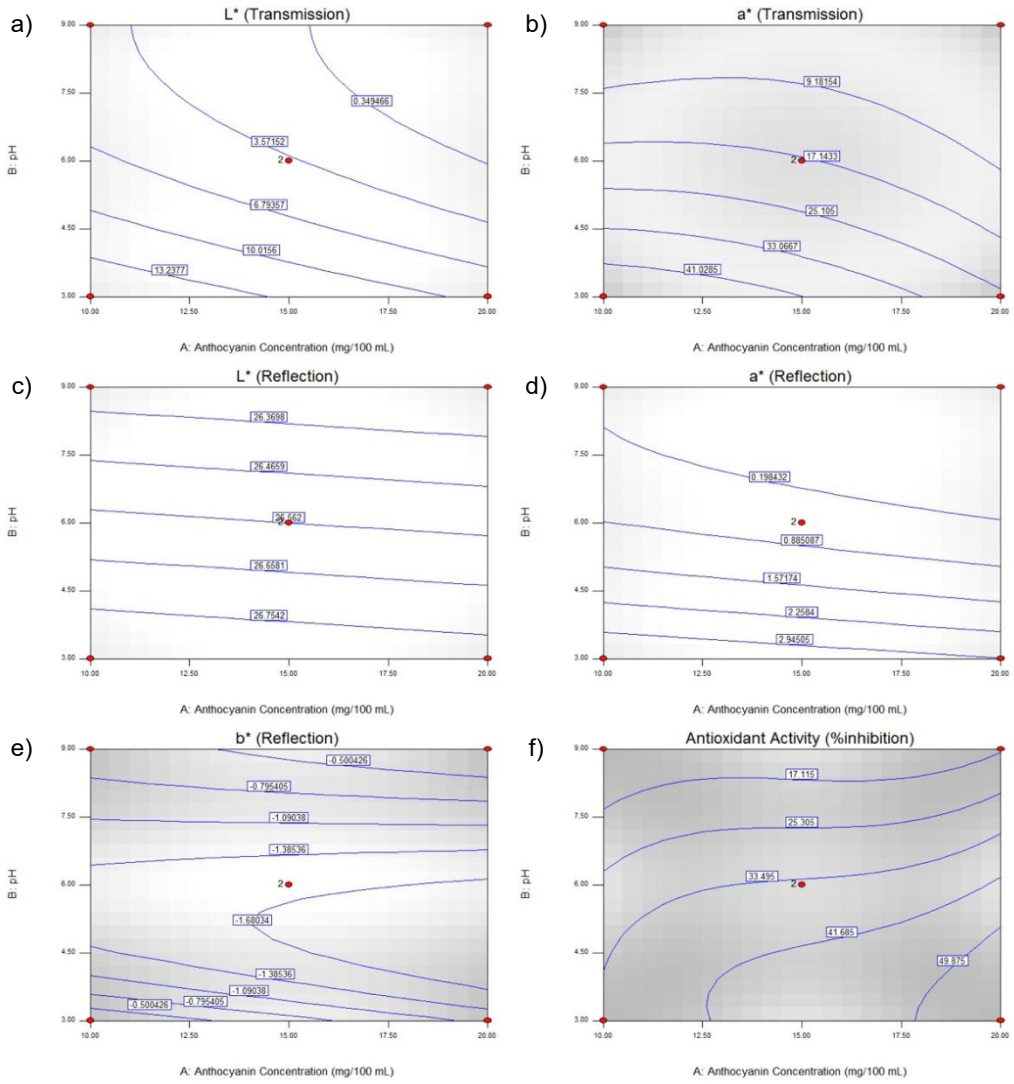

Figure 3. Contour plots of significant parameters: a) color L* (Transmission); b) color a* (Transmission); c) color L* (Reflection); d) color a* (Reflection); e) color b* (Reflection) and f) antioxidant activity (%inhibition).

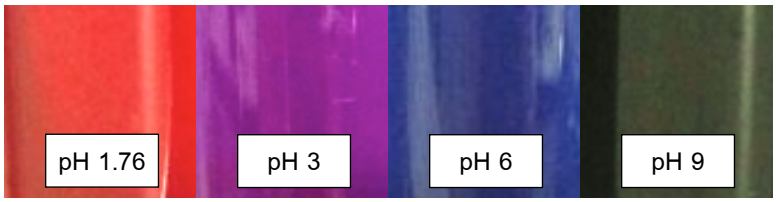

Figure 4. Color change in different pH of *Clitoria ternatea* extract

3.2.2 Effect on Antioxidant Activity

DPPH radical-scavenging activity has been extensively used for screening antioxidants such as polyphenols and anthocyanins (Vareed et al., 2006). DPPH is scavenged by polyphenols and anthocyanins through the donation of hydrogen forming the reduced DPPH. As a result, the color of the solution changes from purple to yellow after reduction which can be quantified by its decrease in absorbance at a wavelength of 515 nm (Hurtado et al., 2009; Sun et al., 2009). Fig. 3f shows the DPPH radical-scavenging activity of anthocyanin extracts from Butterfly Pea petals, which indicate that the main influence on antioxidant activity of the extracts was pH. The coefficients in the regression equation in Table 4 reconfirm the effect of pH which shows high activity at acidic pH and significantly dropped at basic pH. The major reason for this change is that a structure of anthocyanins in acidic pH results in more stable form of reduced anthocyanins by free radicals. This effect was explained in the study of Hurtado et al. (2009) which stated that the present hydroxyl groups in some specific position in the antioxidant molecules could confer higher stability on the formed radical. In particular, they were more stabilized in some structure than others. So, the hydroxyl groups on the molecules of anthocyanins in acidic pH might attach to more stabilizing location in the molecules than anthocyanins in basic solution; accordingly, anthocyanins in acidic solution have higher antioxidant activity.

Even though pH was a predominant factor in determining antioxidant activity of the extract in most range of pH, anthocyanin concentration started to become equally significant to antioxidant activity when the pH is less than 4.5. This effect suggested an insight to improve the antioxidant activity of the extract that antioxidant activity can be improved by adding more anthocyanin content at the optimum pH. Moreover, the result in Fig. 3e and regression equation in Table 4 can not only used to predict the condition yielding highest antioxidant activity but also to predict the color of the resulting extract. Unfortunately, the color acceptance in this study was not significant. If it

was significant, the results would be used to optimize the best condition which pose a highest antioxidant activity while maintain the acceptable consumer likeness for further research in antioxidant beverages. Still, with the current results, all the models can be used in combination to calculate or optimize for the required condition (pH and anthocyanin concentration) that fit to certain color or antioxidant activity criteria. For example, to produce an antioxidant film with color restriction, the models can optimize for the condition which has highest antioxidant activity while its color remains in range.

3.2.3 Optimization of pH and anthocyanin content

In this study, the criteria for optimization were a point where maximum antioxidant activity of the extract was achieved, while other significant parameters were in range of the experiment. The optimization result is shown in Fig. 5. The optimum point yielding maximum antioxidant activity in this study was at pH 3 with anthocyanin content equal to 20 mg/100 mL of the extract resulting in 57.59%inhibition. In fact, the recommended condition for the best antioxidant activity from *Clitoria ternatea* flower extract would be at the pH less than 3 and anthocyanin concentration more than 20 mg/100 mL.

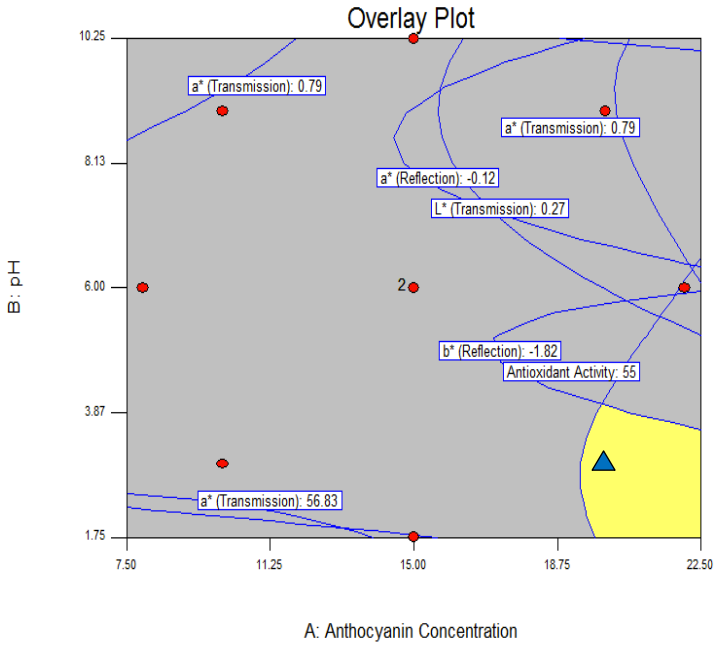

Figure 5. Optimization of anthocyanin concentration and pH (treatment points; ● and optimum point; ▲).

4. Conclusion

The characteristics of color and antioxidant activity of the Butterfly Pea petal crude extracts had been studied in aqueous solution. The relationships of color and antioxidant activity in response with the change of pH and anthocyanin concentration had been significantly proven. While pH value had a major influence in determining the color and antioxidant activity of the extracts, anthocyanin concentration posed a minor influence. The color changes of the extract were red in acidic solution, green in basic solution and blue in neutral solution. In addition, the antioxidant activity showed the high activity in the acidic solution and drastically dropped in the basic solution. All color and antioxidant activity differences might come from the structural changes in anthocyanin molecules responding the change in pH which some forms more stable than the others. The study had provided a preliminary step for further development of *Clitoria ternatea* flower extract into more innovative applications such as antioxidant drinks and intelligent packaging.

Acknowledgements

The authors sincerely acknowledge to Faculty of Agro-Industry, Chiang Mai University for the financial support.

References

Chen, X., and Gu, Z. 2013. Absorption-type optical pH sensitive film based on immobilized purple cabbage pigment. Sensors and Actuators B: Chemical. 178: 207-211.

Chigurupati, N., Saiki, L., Gayser Jr, C., and Dash, A. K. 2002. Evaluation of red cabbage dye as a potential natural color for pharmaceutical use. International Journal of Pharmaceutics, 241(2): 293-299.

Hou, F., Zhang, R., Zhang, M., Su, D., Wei, Z., Deng, Y., Zhang, Y., Chi, J., and Tang, X. 2013. Hepatoprotective and antioxidant activity of anthocyanins in black rice bran on carbon tetrachloride-induced liver injury in mice. Journal of Functional Foods. 5(4): 1705-1713.

Hurtado, N. H., Morales, A. L., González-Miret, M. L., Escudero-Gilete, M. L., and Heredia, F. J. 2009. Colour, pH stability and antioxidant activity of anthocyanin rutinosides isolated from tamarillo fruit (*Solanum betaceum* Cav.). Food Chemistry. 117(1): 88-93.

Kazuma, K., Noda, N., and Suzuki, M. 2003. Malonylated flavonol glycosides from the petals of *Clitoria Ternatea*. *Phytochemistry.* 62(2): 229-237.

Kirca, A., Özkan, M. and Cemeroglu, B. 2007. Stability of black carrot anthocyanins in various fruit juices and nectars. Food Chemistry. 97(4): 598-605.

Li, J., Li, X.-d., Zhang, Y., Zheng, Z.-d., Qu, Z.-y., Liu, M., Zhu, S.-h., Liu, S., Wang, M., and Qu, L. 2013. Identification and thermal stability of purple-fleshed sweet potato anthocyanins in aqueous solutions with various pH values and fruit juices. Food Chemistry. 136(3–4): 1429-1434.

Lin, J.-Y., Li, C.-Y., and Hwang, I. F. 2008. Characterisation of the pigment components in red cabbage (*Brassica oleracea L. var.*) juice and their anti-inflammatory effects on LPS-stimulated murine splenocytes. Food Chemistry. 109(4): 771-781.

Maisuthisakul, P., Suttajit, M., and Pongsawatmanit, R. 2007. Assessment of phenolic content and free radical-scavenging capacity of some Thai indigenous plants. Food Chemistry. 100: 1409–1418.

Mazza, G. and Miniati, E. 1993. Anthocyanins in fruits, vegetables, and grains. Legumes, CRC Press, Boca Raton Inc., Florida. 1-28.

Singh, J., and Tiwari, K. N. 2012. In vitro plant regeneration from decapitated embryonic axes of Clitoria ternatea L.—An important medicinal plant. Industrial Crops and Products. 35(1): 224-229.

Sompong, R., Siebenhandl-Ehn, S., Linsberger-Martin, G. and Berghofer, E. 2011. Physicochemical and antioxidative properties of red and black rice varieties from Thailand, China and Sri Lanka. Food Chemistry. 124: 132–140.

Sun, J., Yao, J., Huang, S., Long, X., Wang, J., and García-García, E. 2009. Antioxidant activity of polyphenol and anthocyanin extracts from fruits of Kadsura coccinea (Lem.) A.C. Smith. Food Chemistry. 117(2): 276-281.

Torskangerpoll, K. and Andersen, O.M. 2005. Colour Stability of Anthocyanins in Aqueous Solutions at Various pH Values. Food Chemistry. 89: 427-440.

Vareed, S. K., Reddy, M. K., Schutzki, R. E., and Nair, M. G. 2006. Anthocyanins in Cornus alternifolia, Cornus controversa, Cornus kousa and Cornus florida fruits with health benefits. Life Sciences. 78(7): 777-784.

Walkowiak-Tomczak, D., and Czapski, J. 2007. Colour changes of a preparation from red cabbage during storage in a model system. Food Chemistry, 104(2): 709-714.

Wangsintaweekul, J. 2549. Color of butterfly pea flower. URL. (http://drug.pharmacy. psu.ac. th/wbfile/1112254516001.doc) (27 September 2556).

Yang, Z., and Zhai, W. 2010. Identification and antioxidant activity of anthocyanins extracted from the seed and cob of purple corn (Zea mays L.). Innovative Food Science and Emerging Technologies. 11(1): 169-176.

Yoshida, C. M. P., Maciel, V. B. V., Mendonça, M. E. D., and Franco, T. T. 2014. Chitosan biobased and intelligent films: Monitoring pH variations. *LWT* - Food Science and Technology. 55(1): 83-89.

Zhou, Z., Nair, M. G., and Claycombe, K. J. 2012. Synergistic inhibition of interleukin-6 production in adipose stem cells by tart cherry anthocyanins and atorvastatin. Phytomedicine. 19(10): 878-881.

Zhu, Y., Ling, W., Guo, H., Song, F., Ye, Q., Zou, T., Li, D., Zhang, Y., Li, G., Xiao, Y., Liu, F., Li, Z., Shi, Z., and Yang, Y. 2013. Anti-inflammatory effect of purified dietary anthocyanin in adults with hypercholesterolemia: A randomized controlled trial. Nutrition, Metabolism and Cardiovascular Diseases. 23(9): 843-849.