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# Thermal Degradation of Blue Anthocyanin Extract of *Clitoria ternatea* Flower

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***Abstract*- Thermal degradation behaviour of anthocyanin extract of *Clitoria ternatea* flower (CTAE) at temperature range of 5–160 $^{o}$C were studied. Effects of benzoic acid and light on its stability at storage temperature were also investigated. Thermal degradation of CTAE showed first order kinetics only at 70, 100 and 160 $^{o}$C. At 5 $^{o}$C, it showed a gradual decrease in color intensity to about 85% over the period of 30 days. At 27–45 $^{o}$C, the CTAE showed a dramatic decrease in stability after a certain "stabilization period" and then leveled off. The length of "stabilization period" was temperature dependent and was in the order of 45>27>37 $^{o}$C. The presence of benzoic acid in the extract lengthened the "stabilization period" at 27 and 37 $^{o}$C. Light caused about 35% reduction of anthocynin color intensity. As blue CTAE has relatively high thermal stability, it has the potential to be used as natural blue colorant for food, cosmetic and pharmaceutical products at pH near neutrality.**

***Keywords-Clitoria ternatea, anthocyanin, thermal degradation, intermolecular complexation, copigmentation***

## I. INTRODUCTION

*Clitoria ternatea* flower is one of our candidates in the studies of floral anthocyanins. *Clitoria ternatea L. Fabaceae* a perennial climber herb, commonly known as butterfly pea, is distributed in tropical countries. It has been implicated to have several medicinal properties [1]. It has several different flower color; namely dark blue, light blue, mauve, and white. The wild blue mauve *Clitoria ternatea* grows widely in Malaysia. The plant bears solitary, axillary papillionaceous bright blue petals flowers with white or light yellow at the center. The bright blue color of the flower extract has been used to dye rice cake in Malaysia and the flowers are eaten as vegetables in Kerala (India) and in the Philippines.

Structural studies of anthocyanins of *Clitoria ternatea* flower petals have been reported. They were found to be mainly ternatins, fifteen of them, which were mainly malonylated delphinidin 3,3'5'-triglucosides [2,3] having 3'5'-side chains with alternative D-glucose and *p*-coumaric acid units and delphinidin 3-O-(2"-O-α-rhamnosyl-6'-O-malonyl-β-glucoside [4-5].

Recently, water soluble anthocyanins have become increasingly important and have received great interests in research as they not only impart beautiful coloration to food systems but also have antioxidant properties and health benefits such as enhancement of sight acuteness, antioxidant capacity, controlling Type II diabetes, reduction of coronary heart disease and prevention of cancer [6-8]. The stability of anthocyanins is correlated with structural features of anthocyanins and is also affected by factors such as heat, pH, light, the presence of enzymes, phenolic acids, oxygen, sugars, sulfur dioxide and metal ions [9-12].

To our knowledge, report on the physicochemical studies of anthocyanins of *Clitoria ternatea* flower is scarce. The pH stability of CTAE has been reported [13]. The objectives of this study were to evaluate the stabilities of blue Clitoria *ternatea* anthocyanin exract (CTAE) at pH 5.8 at temperature range of 5-160 $^{o}$C as well as the effects of benzoic acid and light on its stability at storage temperature.

## II. METHODOLOGY

### A. Materials

Methanol, and hydrochloric acid were purchased from Sigma-Aldrich. Sodium benzoate was obtained from Asia Pacific Chemicals Limited. Malvidin-3-O glucose was purchased from Polyphenols Laboratories AS. All reagents used were of analytical grade and were used without purification.

### B. Preparation of Clitoria ternatea Anthocyanin Extract (CTAE)

The flower petals of *Clitoria ternatea* were harvested from the plant grown in the garden and were cut into small pieces and dried at 50 °C for 24 h. Dry petals (4 g) of *Clitoria ternatea* was extracted with distilled water (240 mL). The suspension was filtered and the solvent of the filtrate was removed using rotary evaporator under vacuum. The dry residue was stored in a refrigerator until use.

### C. UV-Vis Spectroscopy

All UV-Vis measurements were run on UV- Vis spectrophotometer (Perkin Elmer, Lamda 35). UV-Vis spectra of CTAE (0.4 mg/mL) were recorded at wavelengths 200-700 nm.

### D. Temperature Stability Studies

Thermal stability of blue CTAE (0.4 mg/mL) in aqueous solution (pH 5.8) was studied by monitoring the absorbance at the wavelength of maximum absorption ($\lambda_{max}$) at 574 nm of anthocyanins in CTAE. The absorbance value was

directly proportional to the concentration of blue quinonoidal bases of anthocyanins. Ten samples in capped and sealed pyrex tubes were stored in incubators at 27, 37 45, 70, 100 and 160 °C and in a refrigerator at 5 °C for a period of about 30 days or as otherwise indicated. At appropriate time intervals, the samples were taken out to measure the absorbance at 574 nm.

### *E. Effects of Light on the Stability*

Blue CTAE in aqueous solutions (0.4 mg/mL, pH 5.8) were exposed to light with intensity of 600 Lux measured with a light meter (Fortech instrument, 401025) in a room (90' x 30') and another set of samples was kept in the dark in an incubator at 27 °C.

### *F. Effect of Benzoic Acid on the Stability*

Sodium benzoate (0.02%) was added to blue CTAE aqueous solutions (0.4 mg/mL, pH 5.8). The stability of samples at 27 and 37 °C were evaluated as described in section D. Sample without benzoic acid was used as a negative control.

### *G. Statistical Analysis*

Data were studied by analysis of variance (ANOVA); and means were compared using LSD (least significant difference). All comparisons were carried out at a significant level of $P<0.05$.

## III. RESULTS AND DISCUSSION

### *A. UV-Vis Spectrum of Blue CTAE*

UV-Vis spectrum of blue CTAE (2.1 mg/mL) in distilled water (pH 5.8) exhibited wavelength of maximum absorption ($\lambda_{max}$) at 268, 295, 574 and 619 nm and a shoulder at 540 nm. The UV absorption bands at 268 and 295 nm correspond to Band II of the A-ring benzyol system of anthocyanin and also phenolic compounds in the extract while the visible absorption bands correspond to Band I of the B-ring of hydroxyl cinnamoyl system of anthocyanin. The ratio of $E_{440}/E_{573}$ CTAE was estimated to be 43% respectively suggesting that anthocyanins of CTAE are polyglycosylated [10]. The ratio of $E_{310}/E_{573}$ was 236% suggesting that the anthocyanins in CTAE are possibly polyacylated with aromatic acids on the glycosyl residues [10]. The high ratio may also reflect the presence of some free phenolic compounds in the extract. The shoulder at 352 nm may indicate the presence of flavonol glycosides which was found to exist in the extract of *Clitoria ternatea* [5].

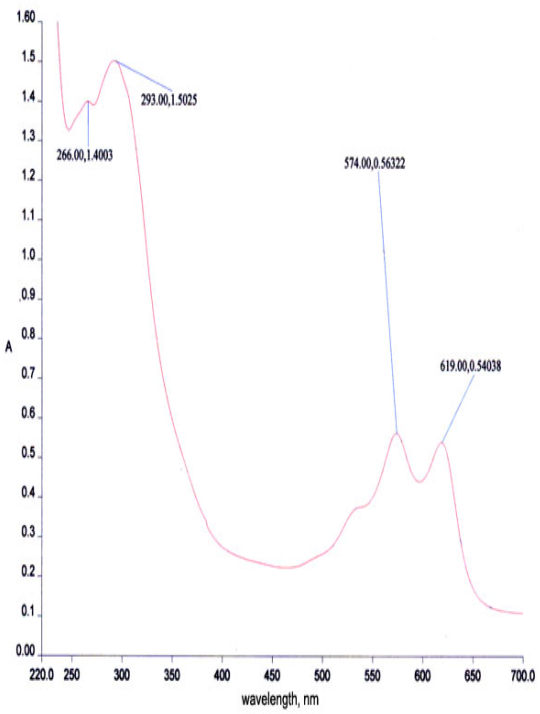


Figure 1. UV-Vis absorption spectrum of CTAE (0.40 mg/mL) in aqueous solution (pH 5.8)

These spectral characteristics of CTAE suggest that the blue CTAE may also contain polyacylated delphinidins, the ternatins, the 3'- and 5'-OH on the B-ring that are substituted with a series of chains with alternating D-glucosyl and p-coumaryl units [3,4] and flavonol glycosides [5,6] as for the *Clitoria ternatea* species found in Japan.

The visible spectrum of blue CTAE may be attributable to the equilibrium mixtures of red flavylium cations appeared as a small shoulder at 543 nm and the two tautomers of neutral blue quinonoidal bases with two absorption bands at 574 and 619 nm. The visible spectral characteristics are also similar to the copigment complexes of acylated and glycosylated delphinidin as reported by Bloor [14]. It is possible that the quinonoidal bases form intermolecular complexes with copigment flavonol glycosides in CTAE as was found in the flower of *Ceanothus papillosus* [14] or complex with copigment of free phenolic acid or flavonol glucoside molecules [15] in the extract. This complexation leads to a big bathochomic shift of the absorption bands from 543 nm to 574 and 619 nm. The intermolecular copigment complex may be formed by the folding over of the glycosyl chain attached with the acylated aromatic ring which is stacking on top of the co-pigment that is lying above the flat anthocyanin chomophore [14-16] Terahara et al. [3] suggested that ternatin of *Clitoria ternatea* in acid solution forms intramolecular complex on the basis of NMR spectral characteristics and Yoshida et al. [16] also reported that

Heaven Blue anthocyanin also forms intramolecular complex. However, it is possible that the blue quinonoiidal bases of blue CTAE forms intermolecular complex.

An experiment was conducted to investigate the possibility of formation of intermolecular co-pigmentation complex of blue CTAE with phenolic compound. A solution of 0.02 % of benzoic acid was added to the blue CTAE solution, a bathochromic shift of the maxima from 619 nm to 624 nm and a hyperchromic effect were observed at the maxima 624 nm (data not shown). This indicates the formation of benzoic acid-anthocyanin co-pigment complex. This is in agreement with the findings of [15] who reported that the intermolecular copigment complex of malvidin with ferulic and coumaric acids led to bathochromic and hyperchromic effect of the absorption bands. Therefore it is postulated that the blue quinonoidal base of CTAE at pH near neutrality is more likely to form intermolecular copigment complex while the flavylium cation would form the intramolecular complex under acidic condition. The absorption band at 619 nm was unlikely to be attributed to the formation of metal quinonoidal complexes as was observed in *Salvia uliginosa* [17] though iron or magnesium were found in the extract (unpublished). This is due to the unavailability of the metal chelating site in the molecular structure of polyglycosylated and polyacylated delphidin or ternatin in CTAE. The above proposition of blue CTAE forming intermolecular copigment complex awaits further studies on CTAE and purified anthocyanin from CTAE.

### *B. Thermal Degradation Studies*

Thermal stability of blue CTAE was studied at the storage temperature range of 5-45 °C as well as at high temperatures of 70-100 and 160 °C, which are the common food processing temperatures for example cooking, pasteurizing, frying, blanching and canning. A common phenomenon in terms of the color variation on heat treatment of blue CTAE solutions from the beginning to the end of the studied period was observed. The color of blue CTAE changed from intense blue to light blue and then bluish green and finally to light yellow color. There was no brown precipitate formation which was mostly found as the final product of thermal studies for anthocyanin extracts of fruits and flowers. The absence of brown precipitate at all temperatures studied may be due to the absence of o-dihydroxy phenolic compounds and polyphenoloxidase [18] in the extract and therefore preclude the formation of the polymeric quinone-anthocyanin condensation products. The initial reduction in color intensity was due to chemical or enzymatic hydrolysis of the glycosidic bonds of anthocyanins in blue CTAE by glycosidase and/or by ring opening of the quinonoidal base to produce the yellowish chalcone. Chalcone may further degrade to aromatic acids or benzaldehyde derivatives [19]. The degradation profiles of blue CTAE at 10-45 $^{o}$C are shown in Fig. 1a.

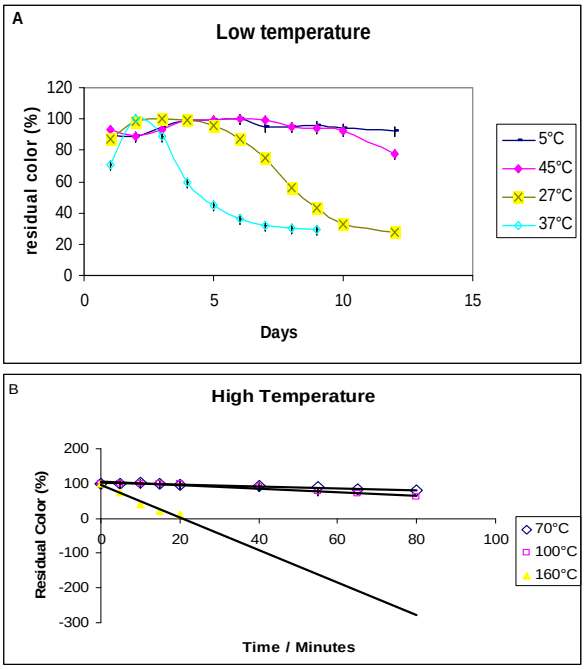


Figure 2. Degradation profiles of CTAE at (A) low temperatures (B) high temperatures

It was found that the blue CTAE showed gradual degradation to about 80% residual color in about 30 days at 5 °C and remained almost unchanged in color intensity for about one year (data unpublished). It did not follow first order kinetics. First order kinetics were also not found for the degradation profiles of CTAE at 27, 37 and 45 °C in contrast to most of the thermal degradation studies of anthocyanin extracts [19] or pure anthocyanins [20]. The degradation profiles showed four stages: an initial slight increase in absorbance followed by a variable "stabilization period" whereby there is a slight reduction in the color intensity. After this so called "stabilization period" of 10, 6 and 10 days at 27, 37 and 45 °C, respectively, a more drastic decrease in absorbance was observed and it was then leveled off. This is a typical reverse sigmoidal curve. It is postulated that the strong intermolecular association of the quinonoidal base with copigment may result in the protection of the degradation of anthocyanins over a short period of time (stabilization period). The thermodynamically stable complex was then dissociated cooperatively to monomeric anthocyanin species as revealed by the reverse sigmoidal curve. The monomeric anthocyanin was more easily susceptible to deglycosylation either enzymatically or chemically producing aglycone which is more susceptible to degradation.

Fig. 1a showed that only about 4% of anthocyanins in CTAE was degraded at 27 $^{o}$C on day 5. However, delphinidin-3-glucoside was found to have lost 70% of its color on day 5. Petanin and cy3-glc completely degraded after 5 and 2 days at 23 °C respectively at pH 6.0–7.0 [18].

Therefore, blue CTAE was relatively more stable than these pure blue anthocyanins at 27 and 37 °C. It was less stable than red cabbage extract which lost 70% of its color at pH 5.0 after 20 days of storage at 20 °C [19]. From Fig. 1a it appears that the degradation of blue CTAE was most rapid at 37 °C ($p<0.05$) suggesting that the degradation process was most likely to be catalysed by enzymes such as glycosidase which was most active at this temperature [18,21] Kircha et al. [22] also observed faster degradation of black carrot anthocyanin extract at 37 °C. It was interesting to observe that CTAE was relatively more stable at 45 °C, a temperature for testing the stability for cosmetic products, compared with those at 27 and 37 °C as typified by a longer stabilization period and greater retention of its color (60%) even after 30 days. This further suggests that the hydrolysis reaction was most likely to be enzyme catalysed as generally glycosidases have optimum activity at 37 °C. This is the reason why the degradation is fastest at 37 °C.

Fig. 1b shows the degradation profiles of blue CTAE solution at high temperatures. It was noteworthy that they followed first order kinetics. This is probably explained by the endothermic dissociation process of the intermolecular co-pigment complex [23] to produce monomeric quinonoidal bases which was then deglycosylated, deacylated and degraded by ring opening of the pyrilium ring to produce yellow aromatic compounds. The kinetic parameters of anthocyanins degradation at these temperatures are summarized in Table 1.

TABLE I KINETIC DATA OF DEGRADATION OF CTAE AT HIGH TEMPERATURES

| Temperature (°C) | $-k \times 10^3$ ($min^{-1}$) | $t_{1/2}$ (min) | *Ea* (kJ/mol) |
|---|---|---|---|
| 70 | 2.2 (0.9122) | 315 | |
| 100 | 9.0 (0.9692) | 77 | 54.56 (0.9988) |
| 160 | 115 (0.9864) | 6 | |

Numbers in parentheses are the determination coefficients

The activation energy for this dissociation and degradation process was 54.56 kJ/mol. The $t_{1/2}$ values of blue CTAE was longer than the period required for high processing temperatures for manufacturing of some food and cosmetics products. In short, the decreasing order of thermal stability of blue CTAE was 5 > 45 > 27 > 37 > 70 > 100 > 160 °C.

### C. *Effect of Benzoic Acid*

Since blue CTAE showed faster degradation at 27 and 37 °C, it is of interest to study the effect of benzoic acid which is often used as a food preservative on the thermal degradation of CTAE at these two temperatures. Fig. 2 shows that the addition of 0.02% of sodium benzoate to blue CTAE enhanced the stability of CTAE by extending the "stabilization period" from 10 to 30 days at 27 °C and from 6 to 12 days at 37 °C. After 25 days at 27 and 37 °C, the samples containing benzoic acid retained about 85% and 25 % of its color respectively. Therefore the blue CTAE solution containing 0.02% benzoic acid is more stable than the blue solution of red cabbage [19]. The increase in stability of anthocyanin in the presence of benzoic acids lends support to our earlier proposition that the blue quinonoidal bases of CTAE could form stable intermolecular copigment complexes with aromatic compounds. The formation of copigment complex creates hydrophobicity on the hydration site of the anthocyanin chomophore and thus enhances the stability of the molecules.

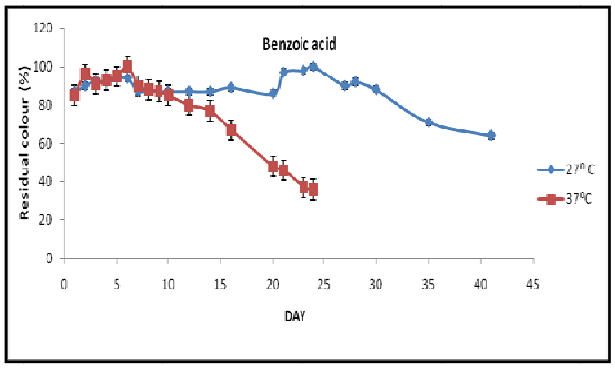


Figure 3. Degradation profiles of blue CTAE in the presence of 0.02% benzoic acid at 27 and 37 oC.

### D. *Effect of light*

As light would cause photodegradation of anthocyanin [19, 24], the effect of light on the stability of blue CTAE was conducted at 27 °C for 12 days. The color of blue CTAE was retained better when kept in the dark. Fig. 3 shows that the stabilization period of the sample kept in the dark was extended by two days and 87% and 53% of blue color remained for the samples kept in the dark and on exposure to the light, respectively after 6 days of storage. This was consistent with the observations reported [20] and that the anthocyanin chromphore was protected from photodegardation by the stacking of the acyl or glycosyl residues of anthocyanins molecules over it. This may account for the stability of blue CTAE towards light exposure.

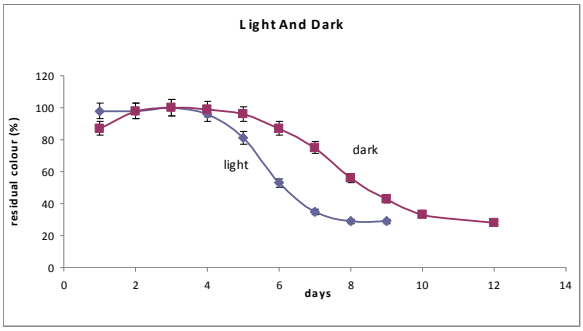


Figure 4. Degradation profiles of blue CTAE on exposure to light and in the dark at 27 oC

## IV. CONCLUSION

This study demonstrated that blue CTAE has great thermal stability and its capability to withstand high temperature for a duration longer than the normal high processing temperature for certain food and cosmetic products. It has a good potential to be used as functional blue colorant in a variety of food, cosmetic, food supplement and pharmaceutical products as it has antioxidant properties [25]. It would be a better natural blue colorant than red cabbage extract as it gives a pleasant taste while red cabbage has off flavor. It is also a good replacement for synthetic blue colorant such as indigiotine, E132 or FD & C Blue No. 2 and Brilliant blue FCF E133 or FD & C Blue No. 1.

This is the first report that the blue CTAE did not follow first order kinetics at 5-45 $^{o}$C and that it is relatively stable at 45$^{o}$C. Involvement of intermolecular complex is postulated in the thermal degradation of blue CTAE at 27 – 45 $^{o}$C. To have a better understanding of the mechanism and kinetics of degradation of blue anthocyanins at various temperatures, further investigation using purified anthocyanins is warranted.

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