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UV-visible light spectra of *Clitoria ternatea* L. flower extract during aqueous extraction and storage

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Abstract

Spectrophotometry is a widespread method to observe anthocyanin content, colour quality, and chemical change of an anthocyanin-source extract. The spectrogram may vary due to the extraction method, and may also change over time. The present work studied the spectrophotometric varieties of butterfly pea flower (*Clitoria ternatea* L.) extract using the cell wall disruption method (mortar-pestle: MP; freezing-thawing: FT; 6 min steam blanching: SB; and 6 min hot water blanching: WB) and aqueous extraction temperature (30, 45, and 60°C) for 210 min. The absorbance was monitored every 30 min. The UV-vis light absorbance shift of the extract during storage at 30°C (E30) and 50°C (E50) was also evaluated. Both SB and WB resulted in extracts with a similar spectrogram and effectively suppressed the brown colour development (browning index: 0.27 ± 0.02 and 0.3 ± 0.01, respectively). The cell wall disruption method did not affect the total anthocyanin. Therefore, the blanching process before extraction was appropriate. The most effective extraction parameter yielding the highest anthocyanin and phenolic substances were 60°C for 30 min. The E50 and E30 performed differently during storage. They showed different colour degradation patterns. For the bathochromic shift, the absorbance increased at 265 nm (phenolic substances), and that at 310 nm (acyl groups) occurred in E50. These three unique characteristics might indicate the event of intermolecular co-pigmentation between or among anthocyanin molecules that led to higher anthocyanin stability at 50°C ($t_{0.5}$ 24.78 days) than at 30°C ($t_{0.5}$ 14.28 days).

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Introduction

The extraction of butterfly pea flower (*Clitoria ternatea* L.) is performed mainly to obtain the blue anthocyanins called ternatins. Ternatins are derivatives of delphinidin glucosides containing one malonic acid at the C3 position. There are nine types of ternatins in the fully opened butterfly pea flower that contains at least two molecules of *p*-coumaric acid at C3 and C5 positions (Kazuma *et al*., 2003). Anthocyanins with two or more aromatic acids configure an intramolecular co-pigmentation. The co-pigmentation protects the hydration of red flavylium cation (AH^+) to colourless hemiketal (B) (Trouillas *et al*., 2016). Therefore, ternatins and several other similar anthocyanins express intense colour in a low acidic solution (Marpaung *et al*., 2019).

Spectrophotometer is a popular device used to study an extract containing anthocyanin. At pH 1, a simple anthocyanin (cyanidin 3-glucoside) exhibits two absorbance bands under a UV-vis spectrophotometer. The first band has λ_{peak} at ± 265 nm, and the second band has λ_{peak} at ± 520 nm (Lee *et al*., 2005). The second λ_{peak} may vary depending on the type of anthocyanin aglycon, the number of glucose molecules, and acyl groups. The absorbance at 265 nm indicates a benzene derivative including anthocyanins (Lee *et al*., 2005). Absorbance at 520 nm belongs to the AH^+, that is, the only species that exist at pH 1. At pH > 5, the peak at ± 520 nm shifts to 550 - 580 nm (purple quinonoidal base, A) or 600 - 620 nm (blue anionic quinonoidal base, A^-). A few anthocyanins, including ternatins, exhibit distinctive characteristics by showing three bands in the visible region (Marpaung *et al*., 2019). The three bands represent all coloured species of anthocyanins. The presence of acyl groups in a polyacylated anthocyanin is marked by a band at ± 310 nm (Wiczkowski *et al*., 2013). The aqueous crude extracts of an anthocyanin-source plant may also contain non-anthocyanin

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flavonoids and other phenolic compounds. Both groups increase the absorbance at ± 265 nm, while non-anthocyanin flavonoids show another band around 350 nm (Chen *et al*., 2012). In total, there are six bands in a butterfly pea flower extract at a low acidic solution.

The compounds in the phenolic family demonstrate different stability to heat and oxidation. Hence, they may undergo different consequences to heat exposure before and during extraction. The storage of the extract at different temperatures may also affect the phenolic compounds that can be observed under a spectrophotometer.

The purpose of the extraction of butterfly pea flowers is not always to gather the colour, but also to obtain the best functional effect of the extract. The common way to realise this objective is by analysing the total anthocyanin, total phenolic content, and antioxidant activity (Marpaung *et al.*, 2013; Mehmood *et al.*, 2019; Pengkumsri *et al.*, 2019). However, those methods are less practical while applying in a series of short period observations. Furthermore, they are unable to reflect the effect of the processing to the acyl group and the occurrence of colour shift in the extract. The acyl group and colour shift are vital to the anthocyanin stability and quality.

The present work thus aimed to spectrophotometrically capture the evolution of benzene derivative, acyl groups, flavonoid, browning development, AH^+, A, and A^- species of ternatins in butterfly pea flower extract during pre-extraction treatment, extraction at several temperatures, and storage at two temperatures. The spectrophotometric data were applied to determine the appropriate extraction parameters, and evaluate the colour evolution of ternatins in butterfly pea extract.

Materials and method

Materials

Fresh butterfly flowers were harvested from a garden in South Tangerang, Banten, Indonesia. The petals were then separated, and treated for cell wall disruption. The chemicals used included potassium chloride (Merck, Germany) and hydrochloric acid (Brataco, Indonesia).

Selecting the method of disruption of cell wall

Four methods to disrupt the cell wall of butterfly pea flower petals were studied—freezing and thawing (FT), applying mechanical force by mortar and pestle (MP), steam blanching (SB), and hot water blanching (100°C) (WB). In the FT treatment, the petals were sealed in a high-density polyethylene (HDPE) plastic bag and stored in a freezer (-20°C) for 2 h. During SB and WB, the petals were sealed in a HDPE plastic bag and blanched for 6 min (Marpaung *et al.*, 2013) using steam or boiling water. Following the treatments, the liquid was mechanically pressed from the petal, and diluted with acidic distilled water (pH 4.5, adjusted by HCl) to a certain dilution factor (DF). Total anthocyanin, browning index, and UV-vis light absorbance of the extract was analysed. The UV-vis light absorbance (200 - 700 nm) was scanned using a spectrophotometer (Genesys 10uv Thermo Electron Corporation, USA). The absorbance at 265, 310, 350, 420, 547, 574, and 620 nm were analysed. The highest total anthocyanin and lowest browning index were the criteria to select the best cell wall disruption method.

Extraction

The cell wall of the petals was disrupted by the selected method. Five grams of the disrupted petals were extracted using 20 mL acidic distilled water (pH 4.5, adjusted by HCl) at three different temperatures: 30, 45, and 60°C under dark conditions for 210 min. The UV-vis light absorbance (200 - 700 nm) was monitored every 30 min. The absorbance at 265, 310, 350, 420, 547, 574, and 620 nm were plotted on a line graph. The line graph was used to determine the best extraction temperature and time.

Stability test

The extraction with the selected time and temperature was applied to the disrupted petals. The extract was filtered through a filter paper, bottled in dark vials, and stored in an incubator at 30 and 50°C for 5 d. The UV-vis light absorbance (200 - 700 nm) was monitored daily. The spectrogram was analysed to determine the light absorbance's significant horizontal and vertical shift.

Determination of total anthocyanin (TA)

The single pH method was applied to determine the total anthocyanin (stated as delphinidin 3-glucoside) (Aishah *et al*., 2013). The extract was added with potassium chloride pH 1, following which the light absorption at λ_{max} and 700 nm were read by the spectrophotometer using Eq. 1:

$$TA\ (mg/L) = ((A_{\lambda max}) - A_{700}) \times MW \times DF \times 1000) / (\varepsilon \times l) \quad \text{(Eq. 1)}$$

where, MW = molecular weight of delphinidin-3-glucoside (465.2 g/mol), DF = dilution factor, ε = molar absorptivity of delphinidin 3-glucoside (29000), and l = cuvette width.

Colour quality

The colour quality included colour intensity (CI), violet index (VI), and browning index (BI) (Cisse *et al*., 2012), and were assessed using Eqs. 2 – 4:

$$CI = (A_{\lambda max} - A_{700}) \times DF \quad \text{(Eq. 2)}$$

$$VI = (A_{580} - A_{700}) / (A_{520} - A_{700}) \quad \text{(Eq. 3)}$$

$$BI = (A_{420} - A_{700}) / (A_{\lambda max} - A_{700}) \quad \text{(Eq. 4)}$$

where, $A_{\lambda max}$ = absorbance at the wavelength of maximum absorbance, A_{580} = absorbance at 580 nm, A_{520} = absorbance at 520 nm, A_{420} = absorbance at 420 nm, and A_{700} = absorbance at 700 nm for haze correction.

Degradation kinetics

The degradation kinetics of the light absorbance at 265, 310, 350, 420, 547, 574, and 620 nm were evaluated using the first-order reaction using Eqs. 5 and 6:

$$A = Ao.e^{-kt} \quad \text{(Eq. 5)}$$

$$t_{0.5} = \ln (2) / k \quad \text{(Eq. 6)}$$

where, A = final absorbance, Ao = initial absorbance, k = constant of degradation rate (d^{-1}), t = storage time (d), and $t_{0.5}$ = half-life (d).

Experimental design and statistical analysis

The selection of the cell wall disruption method was studied by a one-factor design with three replications. The ANOVA and *post hoc* analysis (significance level, α = 0.05) were conducted by Design Expert® version 7.0 (Stat-Ease, Inc. Minneapolis, USA).

The light absorbance during extraction and storage was the average of two replications. The trend of the change of absorbance at 265, 310, 350, 420, 547, 574, and 620 nm was evaluated by regression analysis (significance level, α = 0.05) using Microsoft Excel® (Microsoft 365, Microsoft, Redmond, Washington, USA).

Results and discussion

Effect of cell wall disruption method

Six typical bands between 250 and 700 nm appeared in all butterfly pea petal extracts (Figure 1). They represented phenolic substances (P, ± 260 nm; Lee *et al*., 2005), acyl groups (Ac, ± 310 nm; Wiczkowski *et al*., 2013), non-anthocyanin flavonoids (F, ± 350 nm; Chen *et al*., 2012), AH^+ (± 550 nm), A (± 575 nm), and A^- (± 620 nm; Marpaung *et al.*, 2019), respectively. The cell wall disruption by heat (SB and WB) yielded similar spectrograms. Both methods showed much higher absorbance at around 260 nm (phenolic substances) than the other non-heat methods. It seemed that heat exposure was significant to extract the phenolic substances from the cells more effectively. Several studies also reported more effective extraction of phenolic substances by applying more heat (Xu *et al.*, 2007; Marpaung *et al*., 2013; Kim *et al.*, 2019).

Further quantified characteristics can be demonstrated by calculating the ratio of two important wavelengths like Ac/P (A_{310}/A_{265}), F/P (A_{350}/A_{265}), A/P (A_{574}/A_{265}), A/F (A_{574}/A_{350}), A^-/AH^+, A^-/A, violet index (VI), and Browning index (BI) (Figure 1). All these ratios established the adjacency between SB and WB. The FT and MP were in the other group. Yet, the MP produced an extract with lower VI (more red) and higher BI.

Two criteria were used to select the cell wall disruption method: total anthocyanin (TA) and BI. Although the cell wall disruption by heat yielded higher phenolic substances, the total anthocyanins of all extracts were statistically comparable (Table 1). Hence, the selection of the method has relied on the lowest BI (Cisse *et al.*, 2012). Both blanching methods (SB and WB) effectively suppressed the development of brown colour to yield the lowest BI (Table 1). Mild heating or blanching is the common way to inactivate polyphenol oxidase, an enzyme that is responsible to degrade polyphenols to brown products (Nurhuda *et al.*, 2013; Noreña and Rigon, 2018; Mahmudatussa'adah *et al.*, 2019). There was no significant difference in BI between SB and WB. However, the SB was selected as the method to

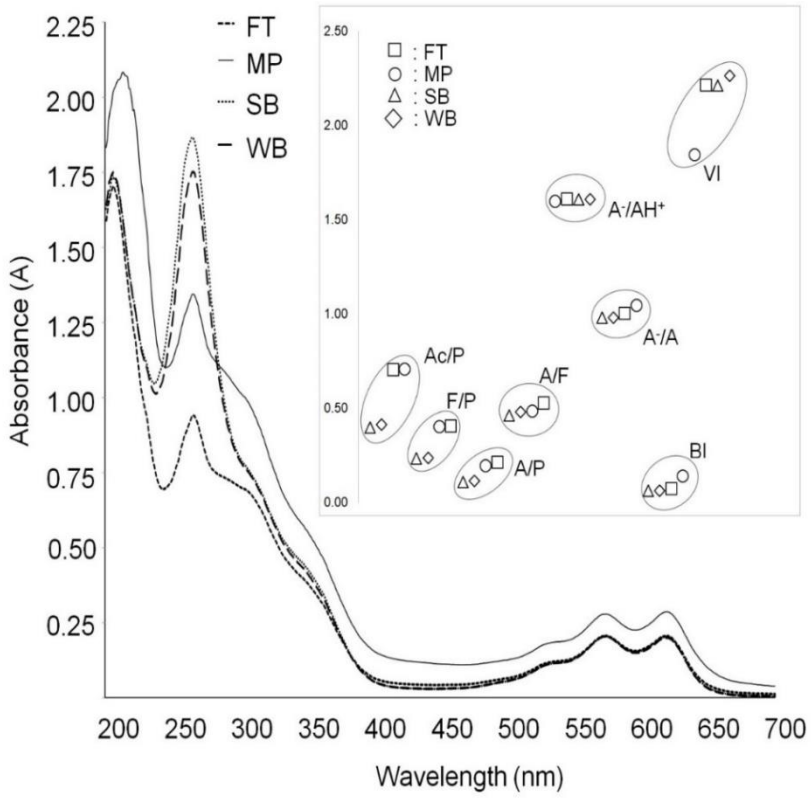

Figure 1. Spectrogram of butterfly pea flower extracts treated by different cell-wall disruption methods. FT: freezing and thawing; MP: mortar and pestle; SM: 6-min steam blanching; and WB: 6-min hot water blanching. VI: violet index; BI: browning index; Ac: absorbance at 310; P: absorbance at 265 nm; F: absorbance at 350 nm; AH+: absorbance at 550 nm; A: absorbance at 580 nm; and A-: absorbance at 620 nm.

Table 1. Total anthocyanin and browning index of butterfly pea flower extract treated by different cell-wall disruption methods.

Disruption method	Total anthocyanin (mg/L)	Browning index
FT	44.43 ± 4.98[a]	0.45 ± 0.02[b]
MP	46.97 ± 2.1[a]	0.75 ± 0.07[a]
SB	51.09 ± 7.25[a]	0.27 ± 0.02[c]
WB	50.43 ± 5.09[a]	0.30 ± 0.01[c]

Means followed by different lowercase superscripts are significantly different at α = 0.05. FT: freezing and thawing; MP: mortar and pestle; SM: 6-min steam blanching; and WB: 6-min hot water blanching.

disrupt the cell wall of the butterfly pea flower. The application of blanching are also chosen to disrupt the cell wall of sour cherry (Gao *et al.*, 2012), blueberry (Jiang *et al.*, 2020), and *Tibouchina semidecandra* flower (Daniella *et al.*, 2021).

Spectrophotometric change during extraction

Figure 2 depicts the absorbance at 265, 310, 350, 420, 550, 580, and 620 nm of the butterfly pea flower extract during extraction at 30, 45, and 60°C for every 30 min. Several results might be concluded from the figure.

First, the stable absorbance at 420 nm indicated the effectivity of steam blanching to inhibit the formation of brown products in the extract.

Second, the extraction at 60°C for 30 min was adequate to extract the anthocyanin from butterfly pea flower. Meanwhile, a longer time would be required if the extraction temperature was set at 30 or 45°C. This result is aligned with a previous study (Marpaung *et al.*, 2013). Therefore, the extraction at 60°C for 30 min was chosen to produce butterfly pea flower extract for stability test.

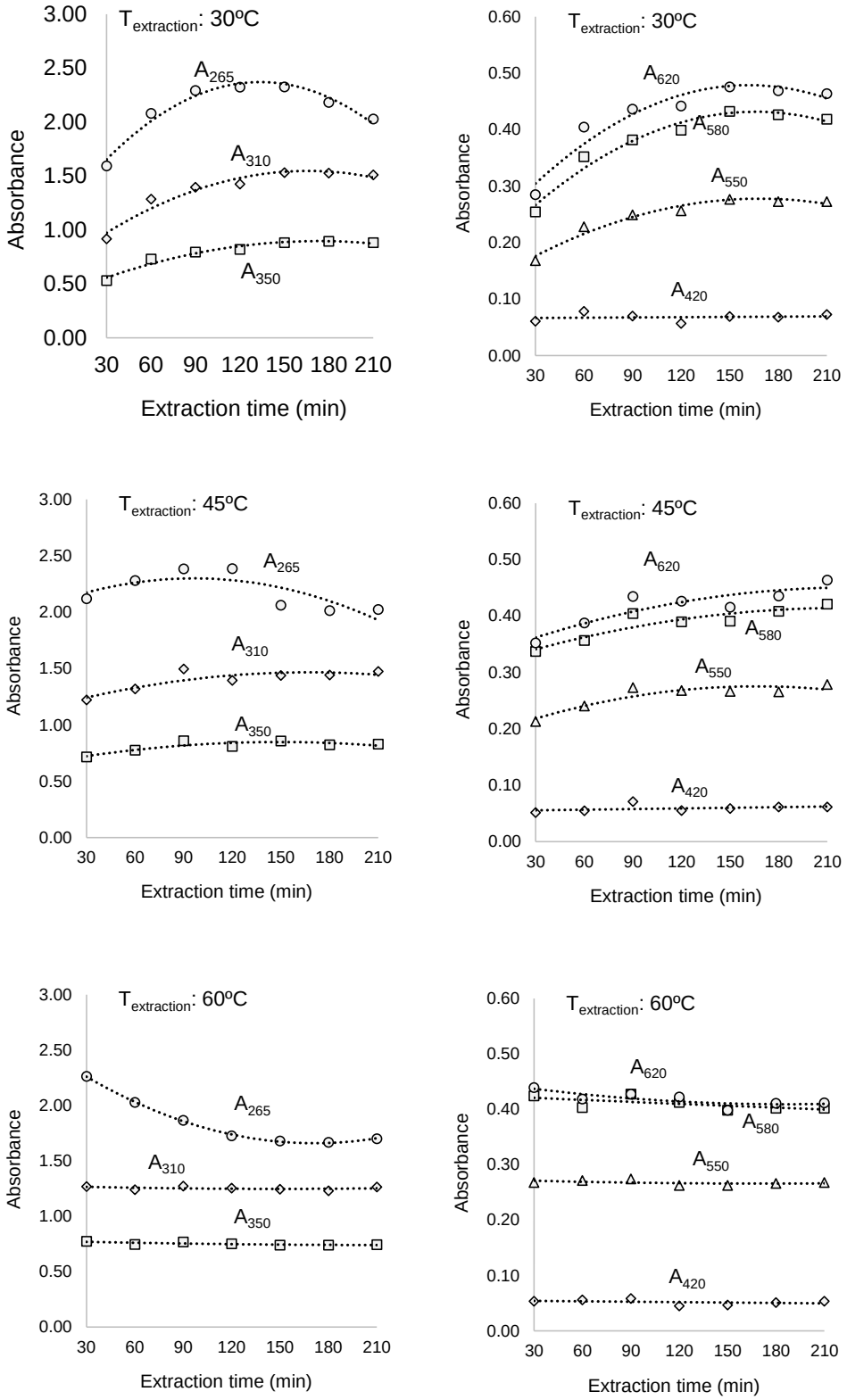

Figure 2. Evolution of light absorbance in butterfly pea flower extracts during extraction at 30, 45, and 60°C.

Third, prolonging the extraction time at 60°C tended to decrease the $A2_{65}$, but it did not affect the other bands. The decrease in A_{265} indicated the degradation of simple phenolic substances. The loss of phenolic substances in various plants due to heat during extraction has been reported by several studies (Shotorbani *et al*., 2012; Mokrani and Madani, 2016).

Fourth and probably the most interesting one, the extraction at 60°C tended to yield more red extract, and the extraction at lower temperature produced more blue extract. This fact resulted from calculating the A_{580}/A_{550} (purple to red ratio) and A_{620}/A_{580} (blue to red ratio) of each extract. The lower A_{580}/A_{550} and A_{620}/A_{580} was showed by the extract extracted at 60°C.

In a low acidic butterfly pea flower extract, three coloured anthocyanins species existed in an equilibrium: red AH^+ ⇌ purple A ⇌ blue A^- (Trouillas *et al.*, 2016). It seemed that 60°C tended to shift the equilibrium to the proton donor reaction: $A^- \rightarrow A \rightarrow AH^+$. This phenomenon opened a possibility of butterfly pea flower extract as a heat indicator. Further research is required to explore this prospect.

Evolution of spectrogram during storage

The butterfly pea flower extract stored at 30°C (E30) and 50°C (E50) for 5 d ended up with different spectrograms (Figure 3). There was no horizontal shift in E30, while a bathochromic shift (3 nm) was observed in E50. The λ_{max} of the extract shifted from 617 to 620 nm after 5-d storage at 50°C. In other words, the butterfly pea flower extract tended to be more blue when stored at 50°C.

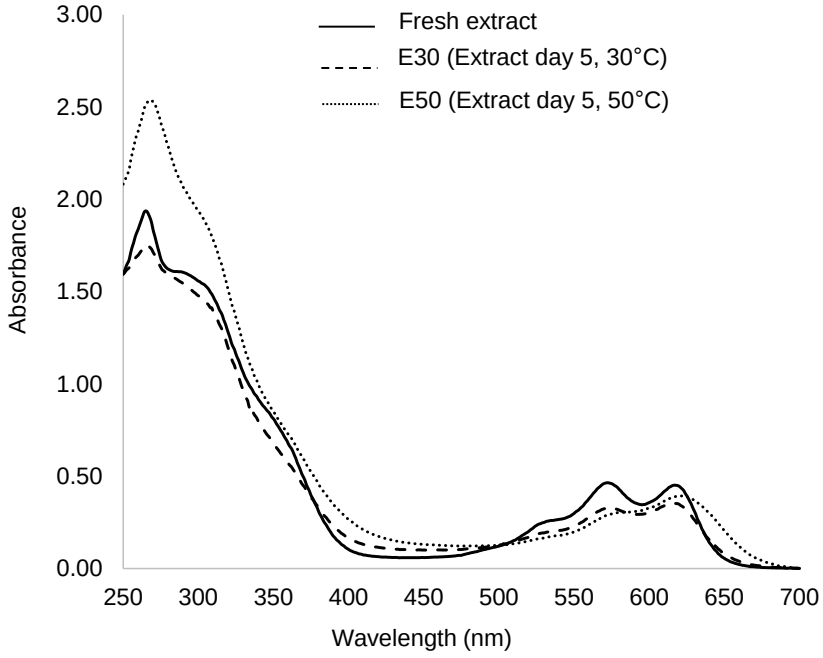

Figure 3. Spectrogram of butterfly pea flower fresh extract after being stored for five days at 30°C (E30) and 50°C (E50). A bathochromic shift appeared in E50.

Previous research reported that the horizontal shift of light absorption of butterfly pea flower extract may occur during storage at room temperature. The shifts depend on the pH of the extract. There was no horizontal shift in the extract at pH 4, while a bathochromic shift occurred in the extract at pH 1, and a hypsochromic shift was observed at pH 7 (Marpaung *et al.*, 2019). Hypsochromic shift is associated with the occurrence of the deacylation of ternatin in butterfly pea flower (Marpaung *et al.*, 2017b; 2019). The present work thus opened another horizon that the horizontal shift of the light absorbance of an anthocyanin extract may also be affected by the storage temperature.

Further study is, nevertheless, needed to explain how the bathochromic shift occurred. Theoretically, a bathochromic shift in an anthocyanin-source extract may happen because of three events. First is the increase of the pH of the extract, which did not occur in the present work. Second is the deglycosylation of the anthocyanin molecules (Marpaung *et al*., 2019), which usually occurred at pH < 2 (Sun *et al*., 2011). The third is the development of complex configuration through co-

pigmentation or metal complexation (Yoshida *et al*., 2009). Butterfly pea flowers contain ternatins with two to four acyl groups per molecule (Kazuma *et al*., 2003). By the chemical structure, the intermolecular interaction through hydrophobic interaction between two or more ternatins may occur (Yoshida *et al*., 2009). The hydrophobic interaction is a thermodynamic event. Hnece, temperature plays an important role.

Figure 4 shows the daily absorbance at 265, 310, 350, 420, 550, 580, and 620 nm of extract E30 and E50. In E30, the pattern of the change of A_{265}, A_{310}, and A_{350} was similar (Pearson's coefficient of correlation, $r > 0.95$). Their decrease was visually observable, but the trend did not fit the zero and first-order degradation kinetic model (Pearson's coefficient of determination, $R^2 < 0.7$ and $p > 0.05$). The E50 showed different characteristics. The A_{265} and A_{310} of E50 increased significantly, and could satisfactorily be modelled by the zero and first-order kinetics reaction ($R^2 > 0.97$ and $p < 0.01$). Meanwhile, the A_{350} remained stable during storage.

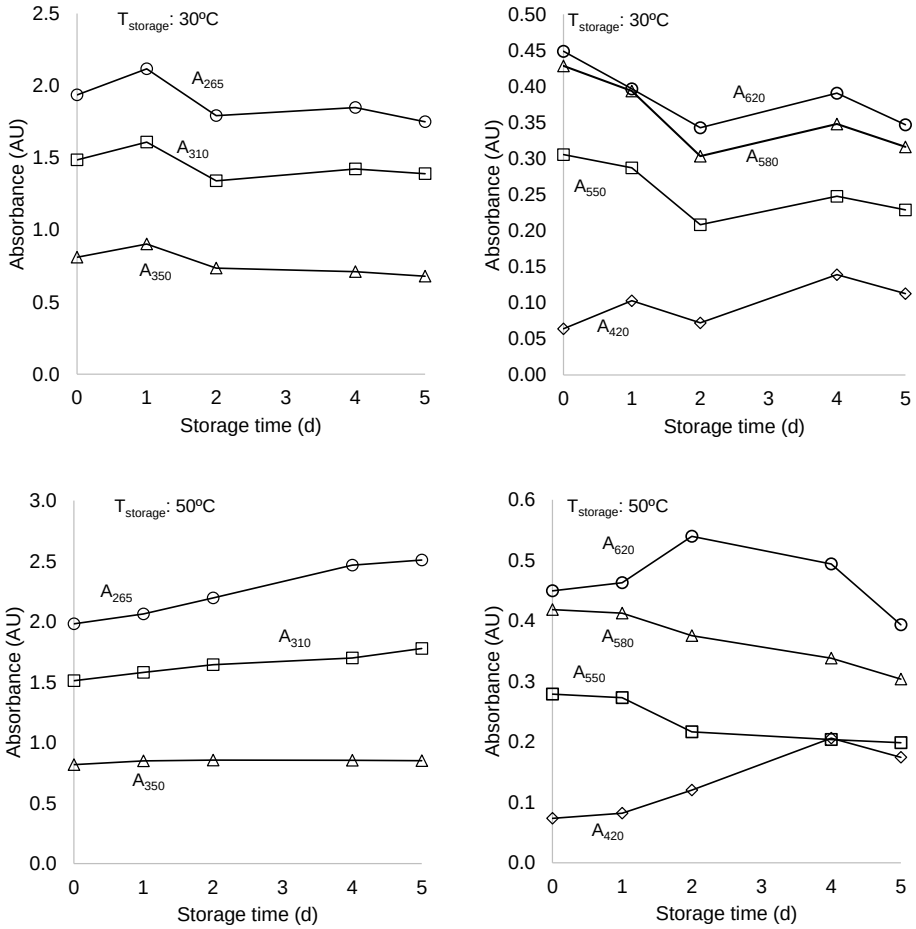

Figure 4. Evolution of light absorbance at 265, 310, 350, 420, 550, 580, and 620 nm (A_{265}, A_{310}, A_{350}, A_{420}, A_{550}, A_{580}, and A_{620}, respectively) in butterfly pea flower extracts during storage at 30 and 50°C.

The A_{265} and A_{310} consecutively indicated the benzene derivatives and acyl groups since both substances absorb light maximally at those wavelengths. However, the increase in absorbance does not necessarily indicate an increase in the number of molecules. On the other side, the λ_{max} of E50 bathochromically shifted during storage (Figure 3). The shift was probably due to the intermolecular interaction between ternatins. Therefore, the increase in A_{265} and A_{310} might be associated with intermolecular interaction.

The fading of red, purple, and blue colours (A_{550}, A_{580}, and A_{620}, respectively) of E30 occurred during storage with a similar pattern ($r > 0.93$). Hence, there was no detectable hue change in the E30. Although the colour fade was observable, their trend did not fit the zero and first-order degradation kinetic model ($R^2 < 0.5$ and $p > 0.05$). There was an up and down pattern of colour intensity of the extract, which indicated the reversible reaction between colourless and coloured species of anthocyanins.

The up and down pattern of colour intensity during storage was absent in E50. The A_{550} and A_{580} consistently decreased, and were best fitted to the first-order degradation kinetics model ($R^2 > 0.85$ and $p < 0.05$). Conversely, the A_{620} increased in the first two days, and then gradually decreased.

The 3 nm bathochromic shift, the increase in A_{265}, A_{310}, and the increase in A_{620} at the initial of the storage of E50 probably described the same event. We propose that the event could be the intermolecular co-pigmentation between or among ternatins, especially as the anionic quinonoidal species (A^-). The intermolecular co-pigmentation between anthocyanins has been reported recently (Zhao *et al.*, 2020).

Total anthocyanin

The stability of E30 and E50 during storage was incomparable based on their colour fading. Therefore, the total anthocyanins of both the extracts were evaluated. The total anthocyanin degradation of both the extracts could be modelled by the first-order degradation kinetics ($R^2 > 0.80$ and $p < 0.05$). The average anthocyanin degradation rate (k) ± standard error of E30 and E50 was 0.0488/d ± 0.0134 and 0.0280/d ± 0.0080, respectively, while the average $t_{0.5}$ was 14.21 and 24.78 d, respectively. In short, the anthocyanin of butterfly pea flower extract at pH 4.5 was more stable at 50°C than at 30°C. A similar result had also been reported by a previous study (Marpaung *et al.*, 2017a). The study showed that the anthocyanin in butterfly pea flower extract at pH 7 was less stable at 30°C than at 45°C.

These results indicated the extra protection of ternatins in butterfly pea against degradation at 45 - 50°C beyond their natural protection through intramolecular co-pigmentation. The extra protection, as we proposed, was probably due to the intermolecular co-pigmentation between or among ternatins.

Conclusion

The observation on UV-visible light absorbance of butterfly pea flower anthocyanin during treatment prior extraction, aqueous extraction, and storage resulted in two groups of conclusions. First, it confirmed the previous research related to the extraction method. The 6-min blanching maintained the colour quality of anthocyanins in butterfly pea flowers before and during extraction effectively. The aqueous extraction (pH 4.5) at 60°C for 30 min was advisable to extract anthocyanins from butterfly pea flowers.

Second, it showed that temperature higher than room temperature affected the colour hue, and might improve the stability of anthocyanin in butterfly pea flowers. The extraction at 60°C shifted the colour to more red. When stored at 50°C for five days, the butterfly pea flower extract showed several unique characteristics, such as the increase in absorbance at 265 and 310 nm (represented phenolic substances and acyl groups, respectively), the increase in absorbance at 620 nm (represented anionic quinonoidal base species of anthocyanin) during the first two days, and a bathochromic shift of λ_{max}. The extract at 50°C also showed higher anthocyanin stability (average $t_{0.5}$ 24.78 d) than at 30°C (average $t_{0.5}$ 14.28 d).

The higher stability at 50°C was most likely due to the intermolecular co-pigmentation between or among anionic quinonoidal base species. The configuration probably increased the absorbance at 265 and 310 nm. However, further study using more appropriate instruments like ^{1}H NMR is required to directly prove the intermolecular co-pigmentation.

References

Aishah, B., Nursabrina, M., Noriham, A., Norizzah, A. and Mohamad, S. 2013. Anthocyanins from *Hibiscus sabdariffa*, *Melastoma malabathricum* and *Ipomoea batatas* and its color properties. International Food Research Journal 20(2): 827-834.

Chen, S. M., Li, C. H., Zhu, X. R., Deng, Y. M., Sun, W., Wang, L. S., … and Zhang Z. 2012. The identification of flavonoids and the expression of genes of anthocyanin biosynthesis in the chrysanthemum flowers. Biologia Plantarum 56: 458-464.

Cisse, M., Vaillant, F., Kane, A., Ndiayea, O. and Dornier, M., 2012. Impact of the extraction procedure on the kinetics of anthocyanin and colour degradation of roselle extracts during storage. Journal of the Science of Food and Agriculture 92: 1214-1221.

Daniella, S., Kartawiria, I. S. and Marpaung, A. M. 2021. Effect of blanching, acid type, and temperature on the extraction of anthocyanin from *Tibouchina semidecandra* flower. Food Research 5(6): 134-140.

Gao, J., Wang, B. G., Feng, X. Y. and Zhu, Y. Q. 2012. Effect of blanching on quality of sour cherry (*Prunus cerasus* L. cv. CAB) juice. American-Eurasian Journal of Agricultural and Environmental Sciences 12(1): 123-127.

Jiang, Q. X., Ning, K. L., Yu, D. W. Xu, Y. S., Wang, B., Yang, F. G., … and Xia, W. S. 2020. Effects of blanching on extraction and stability of anthocyanins from blueberry peel. Journal of Food Measurement and Characterization 14(5): 2854-2861.

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

Kim, M. Y., Lee, B. W., Lee, H. U., Lee, Y. Y. Kim, M. H., Lee, J. Y., … and Kim, H. J. 2019. Phenolic compounds and antioxidant activity in sweet potato after heat treatment, Journal of the Science of Food and Agriculture 99(15): 6833-6840.

Lee, J., Durst, R. W. and Wrolstad, R. E. 2005. Determination of total monomeric anthocyanin pigment content of fruit juices, beverages, natural colorants, and wines by the pH differential method: Collaborative study. Journal of AOAC International 88(5): 1269-1278.

Mahmudatussa'adah, A., Patriasih, R., Maulani, R. R. and Nurani, A. S. 2019. Effect of blanching pre-treatment on colour and anthocyanin of dried slice purple sweet potato (*Ipomoea batatas* L). Journal of Physics: Conference Series 1402: 055080.

Marpaung, A. M., Andarwulan, N. and Prangdimurti, E. 2013. The optimization of anthocyanin pigment extraction from butterfly pea (*Clitoria ternatea* L.) petal using response surface methodology. Acta Horticulturae 1011: 205-211.

Marpaung, A. M., Andarwulan, N., Hariyadi, P. and Faridah D. N. 2017a. Thermal degradation of anthocyanins in butterfly pea (*Clitoria ternatea* L.) flower extract at pH 7. American Journal of Food Technology 5(5): 199-203.

Marpaung, A. M., Andarwulan, N., Hariyadi, P. and Faridah D. N. 2017b. The colour degradation of anthocyanin-rich extract from butterfly pea (*Clitoria ternatea* L.) petal in various solvents at pH 7. Natural Product Research 31(19): 2273-2280.

Marpaung, A. M., Andarwulan, N., Hariyadi, P. and Faridah, D. N. 2019. The difference in colour shifting of *Clitoria ternatea* L. flower extract at pH 1, 4, and 7 during storage. Current Nutrition and Food Science 15(7): 694-699.

Mehmood, A., Ishaq, M., Zhao, L., Yaqoob, S., Safdar, B., Nadeem, M., … and Wang, C. 2019. Impact of ultrasound and conventional extraction techniques on bioactive compounds and biological activities of blue butterfly pea flower (*Clitoria ternatea* L.). Ultrasonics Sonochemistry 51: 12-19.

Mokrani, A. and Madani, K. 2016. Effect of solvent, time and temperature on the extraction of phenolic compounds and antioxidant capacity of peach (*Prunus persica* L.) fruit. Separation and Purification Technology 162: 68-76.

Noreña, C. Z. and Rigon, R. T. 2018. Effect of blanching on enzyme activity and bioactive compounds of blackberry. Brazilian Archives of Biology and Technology 61: 1-13.

Nurhuda, H. H, Maskat, M. Y., Mamot, S., Afiq, J. and Aminah, A. 2013. Effect of blanching on enzyme and antioxidant activities of rambutan (*Nephelium lappaceum*) peel. International Food Research Journal 20(4): 1725-1730.

Pengkumsri, N., Kaewdoo, K., Leeprechanon, W., Sivamaruthi, B. and Sundaram, N. 2019. Influence of extraction methods on total phenolic content and antioxidant properties of some of the commonly used plants in Thailand. Pakistan Journal of Biological Sciences 22(3): 117-126.

Shotorbani, N. Y., Jamei, R. and Heidari R. 2012. Antioxidant activities of two sweet pepper *Capsicum annuum* L. varieties phenolic extracts and the effects of thermal treatment. Avicenna Journal of Phytomedicine 3(1): 25-34.

Sun, J., Bai, W., Zhang, Y., Liao, X. and Hu X. 2011. Identification of degradation pathways and products of cyanidin-3-sophoroside exposed to pulsed electric field. Food Chemistry 126: 1203-1210.

Trouillas, P., Sancho-García, J. C., De Freitas, V., Gierschner, J., Otyepka, M. and Dangles, O., 2016. Stabilizing and modulating color by copigmentation: Insights from theory and experiment. Chemical Reviews 116: 4937-4982.

Wiczkowski, W., Szawara-Nowak, D. and Topolska, J., 2013. Red cabbage anthocyanins: Profile, isolation, identification, and antioxidant activity. Food Research International 51(1): 303-309.

Xu, G. H., Ye, X. G., Chen, J. C. and Liu, D. H. 2007. Effect of heat treatment on the phenolic compounds and antioxidant capacity of citrus peel extract. Journal of Agricultural and Food Chemistry 55(2): 330-335.

Yoshida, K., Mori, M. and Kondo, T. 2009. Blue flower colour development by anthocyanins: From chemical structure to cell physiology. Natural Product Reports 26(7): 884-915.

Zhao, X., Ding, B. W., Qin, J. C., He, F. and Duan, C. Q. 2020. Intermolecular copigmentation between five common 3-O-monoglucosidic anthocyanins and three phenolics in red wine model solutions: The influence of substituent pattern of anthocyanin B ring. Food Chemistry 26(17): 126960.