Research Article

DOI: 10.22146/ifnp.52037 https://journal.ugm.ac.id/ifnp

ISSN 2597-9388

Indonesian Food and Nutrition Progress

ANTHOCYANIN FROM *BAUHINIA PURPUREA* FLOWER: EXTRACTION, COLOR CHARACTERISTICS AND STABILITY

Abdullah Muzi Marpaung[1)*], Darinda Pusha Djani[1)], Della Rahmawati[1)]

[1)] Food Technology Department Swiss German University, Tangerang
[*)] Correspondence Email: abdullah.muzi@sgu.ac.id

ABSTRACT: The determination of the best temperature (45, 60, and 75°C) and time (30, 75, and 120 minutes) to extract the anthocyanins from *Bauhinia purpurea* (BP) flower powder was studied. The anthocyanin was extracted using 40 ml of 1 N HCl/g powder with continuous shaking. Based on the total monomeric anthocyanin (TMA), the best extraction temperature and time were 45°C and 30 minutes, respectively. The BP extract exhibited relatively high red color intensity (CI) at pH ≤ 3 and almost colorless at pH 4 – 6. Meanwhile, at pH ≥ 7, the color was unsuitable for food applications due to the high browning index. Hence, the best performance of BP extract was at pH 3. At this pH, the stability of BP extract was relatively high with the $t_{1/2}$ of CI and TMA at 30°C was 70.30 and 72.89 days, respectively. Nevertheless, the extract was sensitive to heat as represented by the high activation energy (E_a). The E_a of CI and TMA was 64.50 kJ.mol^{-1} and 90.19 kJ.mol^{-1}, respectively. The study showed the potential of BP extract as a red food colorant at pH 3 processed by relatively mild heat treatment.

Keywords: anthocyanins, *Bauhinia purpurea*, color, extraction

INTRODUCTION

Anthocyanins are a large group of polar polyphenol compounds widely distributed in plant in various color ranging from pink to blue. Therefore, it was promoted as the most potential water-soluble natural food colorant (Marpaung et al. 2017). The biggest challenge of anthocyanins as food colorant is their low stability. Besides their chemical structure, temperature and pH are the most affecting factors.

An anthocyanin, at least, consist of one molecule of anthocyanidin and one molecule of sugar. The most common anthocyanidin found in plant is cyanidin, delphinidin, pelargonidin, peonidin, malvidin and petunidin. They differ in the number of hydroxyl and methoxyl groups located at the B-ring. In general, both methoxyl and hydroxyl groups of anthocyanidin tend to decrease the stability of anthocyanin (Cisse et al. 2011). The order of anthocyanidin from the less hydroxyl and methoxyl groups is pelargonidin < cyanidin = peonidin < delphinidin = petunidin = malvidin. An interesting work by Wesche-Ebeling & Montgomery (1990) showedthat cyanidin directly reacted with polyphenol oxidase, but pelargonidin did not. Therefore, the pelargonidin based anthocyanins tend to have a higher stability than the cyanidin based anthocyanin.

Butterfly tree or purple orchard tree (*Bauhinia purpurea*) is widely used in traditional medicine (Shreedhara, et al. 2009). It produces purple-pink flower that has been consumed as a side dish with rice, or to season fish and meat (Orwa et al. 2009). It contains two types of anthocyanin: pelargonidin 3-glucoside, and pelargonidin 3-triglucoside (Shantha, et al., 2015). Other report stated that the *Bauhinia purpurea* (BP) flower contains malvidin 3-glucoside and a small amount of malvidin 3-diglucoside (Beale et al. 1941). Because of the poor stability of most anthocyanins in a low acidic condition, it is common to use the acidified water (pH ≤ 3) as the solvent to extract anthocyanins from their natural matrices. The use of 0.1 N hydrochloric acid at 60°C for 30 minutes to extract anthocyanins from BP flower was reported (Marpaung et al. 2017). However, anthocyanins are relatively unstable. The excessive heat exposure during extraction, especially at very low pH, may destruct the anthocyanin. Sun et al. (2011) reported that at a high acidic condition anthocyanins were hydrolyzed to anthocyanidins and end up as benzaldehyde and benzoic acid derivatives. Hence, the effect of total heat exposure because of the temperature and time of extraction to the anthocyanin of BP is needed to be studied. In addition, the stability test of the extract at various pH is needed to reveal the potency of anthocyanins from BP as a food colorant.

MATERIALS AND METHODS

Materials

The fresh bloomed flower petals were obtained from a garden in Tangerang, Banten, Indonesia. The petals were steamed-blanched for 6 minutes, dried at 45°C (Oven Gravity WiseVen ® WON – 305 High Clean Air Oven) for 24 hours, and grinded to a powder form with particle size of less than 250 µm. The dried powder was stored and closed tightly in a glass bottle and stored in a freezer (-20°C). The hydrochloric acid, sodium hydroxide, buffer solution pH 1 (potassium chloride and hydrochloric acid) 3, (acetic acid-sodium acetate), and pH 4.5 (sodium acetate and hydrochloric acid), gallic acid, Folin-Ciocalteu reagent (FCR), sodium carbonate (Na_2CO_3), ethanol 96% were obtained from Merck® (Germany). The 2,2-diphenyl-1-picrylhydrazyl (DPPH) was purchased from Sigma-Aldrich (Germany). All reagents were analytical grade with no further purification.

Methods

The Extraction of Anthocyanins from BP Flower

The powder was extracted using 1 N hydrochloric acid. The solvent to powder ratio was 40:1 (v/w). The extraction

was performed at various temperature (45, 60, 75°C) and time (30, 75, 120 minutes) in a water bath shaker, with no light. The extract was then filtered and centrifuged at 5488 g in 4°C for 5 minutes. The evaluated parameter were total monomeric anthocyanin, antioxidant activity and total phenolic content.

Total Monomeric Anthocyanin (TMA)

PH differential method was used to determine the TMA (Marpaung et al., 2013). A 0.1 ml extract was mixed with each 0.9 ml buffer solution pH 1.0 and 4.5. The light absorbance at 520 and 700 nm was measured using UV-Vis Spectrophotometer (Genesys 10uv, Thermo Electron Corporation, USA). The difference between absorbencies (A) was determined using this following equation $(A_{520} - A_{700})_{pH1.0} - (A_{520} - A_{700})_{pH4.5}$. The TMA in mg/L was calculated by (A × MW × DF × 1000)/(ε × l). MW was molecular weight of pelargonidin 3-glucoside ((449.2 g/mol), DF was the dilution factor, ε was molar absorptivity (26900), and l was the width of cuvette.

Total Phenolic Content (PC)

The PC determined by Folin-Ciocalteu method and stated as gallic acid equivalent (GAE) (Marpaung et al. 2013). A 0.1 ml extract was mixed with 0.4 ml of Na_2CO_3 (20%) and 0.5 ml FCR and kept for an hour. The absorbance was read at 765 nm. The 0.2 ml gallic acid at elevated concentration (50, 100, 125, 150 and 200 mg/l) was reacted with 0.8 ml of 20% Na_2CO_3 and 1 ml FCR to develop a calibration curve. The PC (mg/L GAE) was equal to Abs/m. Abs was the absorbance (A), and m was the slope of the standard curve of gallic acid.

Antioxidant Activity (AA)

The AA was determined using DPPH method (Marpaung et al. 2013). 11 mg DPPH was added to 9 ml ethanol, then diluted in different concentrations. For measurements, 0.1 ml of sample, 0.75 ml of ethanol and 0.15 ml of DPPH solution were mixed thoroughly. The blank sample was mixture of 0.9 ml ethanol and 0.1 ml of the sample. The control consisted of 0.1 ml distilled water, 0.75 ml ethanol, and 0.15 ml DPPH solution. All were let for about 30 minutes. The light absorbance was measured at 515 nm. The scavenging effect of DPPH or the AA was determined as follow:

$$AA = \frac{Ac - As}{Ac} \times 100\%$$

Ac and As was the absorbance of the control and sample, respectively.

Color Characteristics

The extract obtained from the best extraction process was adjusted to pH 1 to 12 using hydrochloric acid and sodium hydroxide. The λ_{max}, color hue, color intensity (CI), and browning index (BI) of the extracts were evaluated spectrophotometrically.

The CI was calculated as $(A_{\lambda max} - A_{700})$ x DF and the BI as $(A_{430}-A_{700})/ (A_{\lambda max} - A_{700})$ (Marpaung et al. 2015). $A_{\lambda max}$ was the absorbance at the wavelength where the absorbance was maximum, A_{430} and A_{700} was the absorbance at 430 and 700 nm, respectively.

Stability Test

The best extract was adjusted to pH 3 by a buffer solution (citric acid/sodium hydroxide/hydrogen chloride) with DF of 7. The extract was bottled in a dark vial and stored at 30, 45, and 60°C for 7 days with no light. The TMA and CI of the extracts were determined daily.

Degradation kinetics and Activation Energy (Ea)

The kinetic of degradation of TMA and CI were determined by the first order reaction.

$$A = Ao.e^{-kt}$$

Ao was the initial TMA or CI, A was the final TMA or CI, k was degradation rate constant (day^{-1}), t was the duration of storage (day).

The Ea was determined by Arrhenius Equation as follow:

$$k = k_o . e^{(-Ea/RT)}$$

k was the degradation rate, ko was a pre-exponential factor, Ea was activation energy ($j.mol^{-1}$), R was the ideal gas constant (8.3145 $j.mol^{-1}.K^{-1}$), and T was absolute temperature (K).

Statistical Analyses

Two-ways ANOVA (Design Expert® 7.0 software, Stat-Ease, Inc.) and trend analyses (Pearson's correlation and regression analysis) (Microsoft Excel® 2010 software, Microsoft Corporation) were used in the research. The significant level of all analyses was set at α= 0.05. The graph made by Design Expert® 7.0 and modified by Microsoft Powerpoint® software.

RESULT AND DISCUSSION

The Best Extraction Process

The three levels of time and temperature (30, 75, 120 minutes, and 45, 60, 75°C respectively) were applied to obtain the best extraction process. Both factors were significant to affect the total monomeric anthocyanin (TMA) and total phenolic (PC) of the extract at α = 0.05. There was also a significant effect of the interaction between time and temperature. The temperature also significantly affected the total antioxidant activity (AA), but the effect of time was not significant. As seen in Figure 1, the best extraction process to reach the highest TMA and PC were at 45°C for 30 minutes. It was interesting that the application of relatively mild heat treatment was sufficient to extract the anthocyanin from BP powder. As a comparison, the extraction of anthocyanins from roselle was conducted at 100°C for 30 minutes (Cisse et al. 2011), while for the butterfly pea anthocyanin the extraction at 60°C for 30 minutes was applied to obtain the best result (Marpaung et al., 2013). This could probably due to the difference in the solvent used. In both previous research, the solvent was water, while in this research the solvent was 1 N HCl that has a stronger ability to destruct the plant cell

walls. The increase of heat tended to decrease both TMA and a phenolic compound which indicated that the phenolic substance in BP extract was sensitive to heat. In general, anthocyanin and phenolic compound could degrade faster at high temperature (Patras et al. 2010).

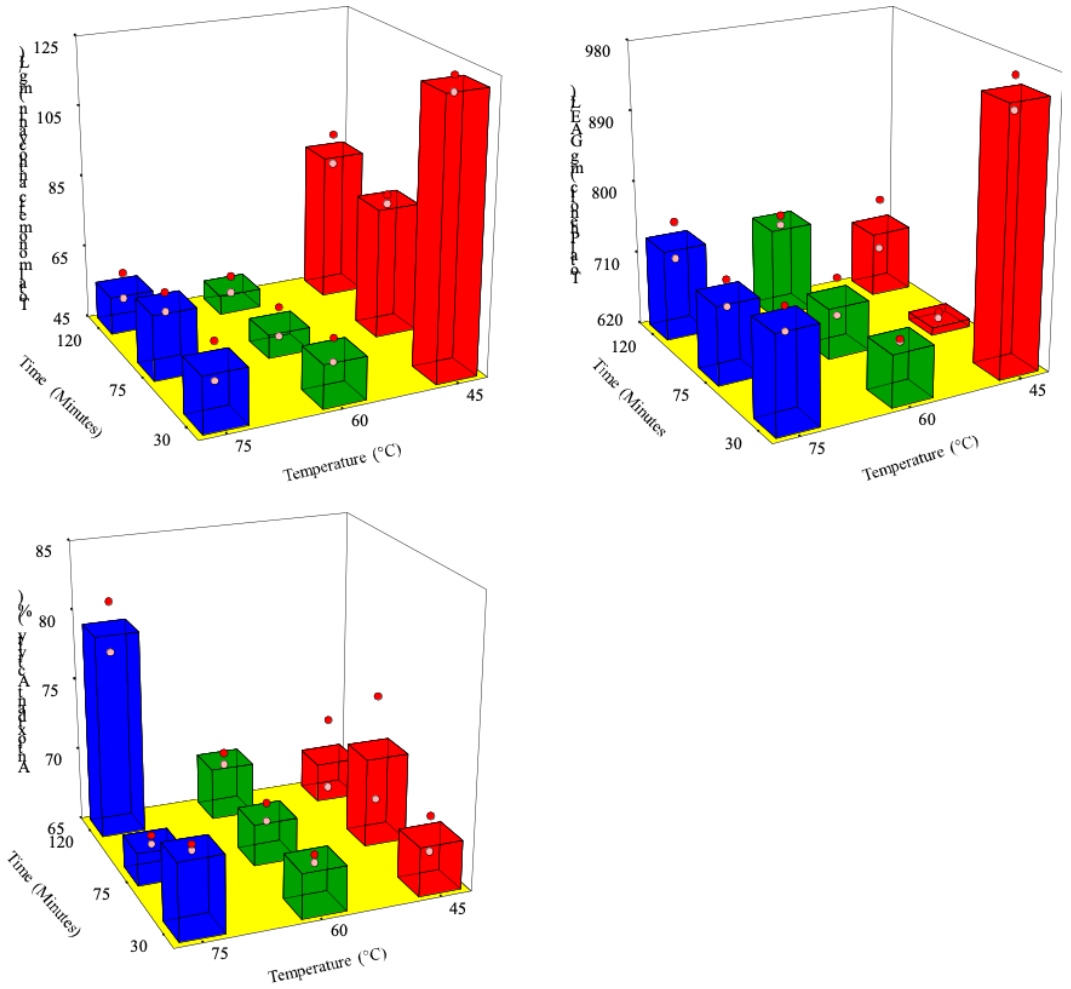

Figure 1. Total monomeric anthocyanin, phenolic content and antioxidant activity of BP flower extracted at different time and temperature.

In contrast to the total anthocyanin and phenolic compound, the highest AA was obtained by extracting the flower with the most severe heat application (75°C for 120 minutes). This could probably due to the contribution of the non-phenolic compound that also exhibited AA. The non-phenolic compound could probably develop during the extraction or extracted by the heavier heat treatment. This was in aggreement with Saric et al. (2013) that heat treatment might increase the AA caused by the Maillard reaction products.

The purpose of the research was to determine the potentiality of BP extract as natural colorant. Therefore, the related parameter to choose the best extraction process was the TMA . As seen in Figure 1, that also supported by the statistical analysis, the chosen time and temperature of the extraction was 30 minutes and 45°C, respectively.

Color Characteristics of BP extract at pH 1 to 12

The color characteristics of BP extract at various pH were similar to the most anthocyanin-source extract like *Melastoma malabathricum*, *Tibouchina semidecandra*, and *Clidemia hirta* (Marpaung et al. 2017). Anthocyanin existed as different species with different color depending on the pH. At pH 1, anthocyanin predominantly presents as red flavylium cation **AH**$^+$. In BP extract, the **AH**$^+$ appeared at 522 nm. As pH increased, **AH**$^+$ gradually hydrated to colorless hemiketal **B**. Therefore, the color intensity (CI) of the extract gradually lowered and reached the lowest CI at pH 5 to indicate the complete hydration of **AH**$^+$ to **B** (Figure 2). At pH 6, the intensity started to increase and the color changed to purple which indicated by the shift of λ_{max} from 522 to 559 nm. The bathochromic shift (the shift of λ_{max} to a longer wavelength) indicated the deprotonation of **AH**$^+$ to quinonoidal base **A.** Figure 2 exhibited that the deprotonation was completed at pH 10. Unfortunately, along with the formation of **A** the pale yellow chalcone **C** was also developed that represented by the increase of BI. As a result, the extract was not purple, but brownish green. The higher BI indicated the lower quality of the color.

Thermal Stability of BP Extract at pH 3

The evaluation of CI and BI of BP extract at pH 1 to 12 showed that the extract performed the best color at pH ≤ 3.

However, pH ≤ 2 was too low for a food product. Therefore, the use of BP extract as food colorant was exclusively potential at pH 3. At that pH, BP extract performed relatively high color stability at 30°C by showing 70.30 and 72.89 days half-life ($t_{1/2}$) of CI and monomeric anthocyanin, respectively (Table 1). This $t_{1/2}$ was much higher than the $t_{1/2}$ of anthocyanins obtained from many sources. At pH 3 and 30°C, the $t_{1/2}$ of anthocyanin from grape, blackberry, black rice, purple potato, and red potato were 31.59, 5.62, 0.13, 22.02, and 55.36 days, respectively (Wang & Xu 2007; Reyes & Cisneros-Zevallos 2007). The higher stability of BP extract could probably because of the difference in the anthocyanidin type. The anthocyanidin in BP extract was pelargonidin which was reported to have higher stability than cyanidin (Bakowska-Barczak 2005).

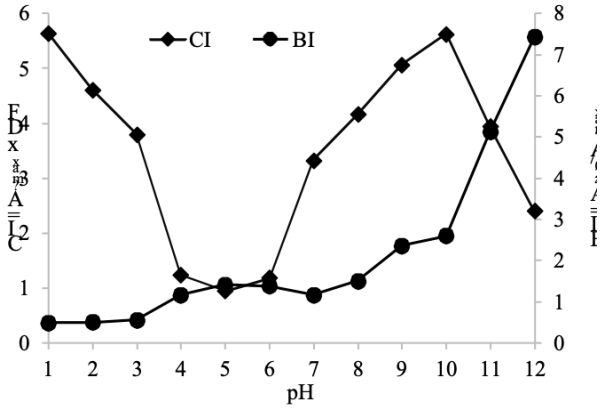

Figure 2. Color intensity (CI) and browning index (BI) of BP flower extract at pH 1 to 12.

Table 1: Degradation parameter of BP flower extract at pH 3.

Parameter	30°C		45°C		60°C		E_a (kJ.mol^{-1})
	Degradation rate, k (day^{-1})*	$t_{1/2}$ (day)	Degradation rate, k (day^{-1})*	$t_{1/2}$ (day)	Degradation rate, k (day^{-1})*	$t_{1/2}$ (day)	
CI	0.0099 ± 0.0075	70.30	0.0411 ± 0.0070	16.85	0.0981 ± 0.0158	7.07	64.50
TMA	0.0095 ± 0.0072	72.89	0.0635 ± 0.0200	10.91	0.2370 ± 0.0431	2.92	90.19

The sensitivity of a bioactive substance like anthocyanin to the heat is commonly represented by the activation energy (E_a). The higher the E_a the more susceptible the anthocyanin to degradation on elevated temperatures. It was fascinating that the E_a of monomeric anthocyanin was much higher than the E_a of CI. It is possible the anthocyanins are more heat dependent and easily degraded, but still, retaining the color in the form of anthocyanidins (the anthocyanin aglycon) which still producing color that remained stable because of the acidic conditions.

The Ea of anthocyanin in BP extract was relatively higher than the E_a of the other anthocyanin source extracts. As comparison, the E_a of anthocyanin extracted from grape, blackberry, black rice, purple potato, and red potato were 75.33, 58.95, 67.48, 72.55, and 67.67 kJ.mol^{-1} respectively ((Wang & Xu 2007; Reyes & Cisneros-Zevallos 2007). The relatively high E_a indicated that the BP extract was not an appropriate candidate as the coloring agent for the product exposed by heat, both during processing and storage.

CONCLUSION

The best temperature and time of extraction to yield the highest anthocyanin from butterfly tree (*Bauhinia purpurea*) flower was 45°C and 30 minutes, respectively. The extract showed a promising performance as the red-pink food colorant at pH 3 by performing a relatively high color intensity and stability at 30°C. However, the stability was rapidly decreased because of the heat treatment. Hence, the best application of *Bauhinia purpurea* flower extract as the coloring agent of food product with pH around 3 and stored at room temperature, such as fruit juices and gelatin desserts.

REFERENCES

Bakowska-Barczak, A. 2005. Acylated anthocyanins as stable, natural food colourants - a review. Polish Journal of Food Nutrition and Science. 14: 107-116.

Beale, G. H., Price, J. R. and Sturgess, V. C. 1941. A Survey of Anthocyanins. VII. The Natural Selection of Flower Colour. Proceedings of The Royal Society B. 130: 113-126.

Cisse, M., Vaillant, F., Kane A., Ndiayea, O. and Dornier, M. 2011. Impact of the extraction procedure on the kinetics of anthocyanin and colour degradation of roselle extracts during storage. Journal of the Science of Food Agriculture. 92: 1214–1221.

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. 2015. Spectral Characteristics and Color Stability of Melastomataceae and *Clitoria ternatea* L. Extracts. *17th Food Innovation Asia Conference.* Bangkok.

Marpaung, A. M., Andarwulan, N., Hariyadi, P. and Faridah, D. N. 2017. Study on Spectral Characteristics of Selected Unacylated, Monoacylated and Polyacylated Anthocyanin Source Plant Extract at Various pH. 15th ASEAN Conference on Food Science and Technology. Ho Chi Minh City. 234-240.

Orwa, C., Mutua, A., Kindt, R., Jamnadass, R. and Anthony, S. 2009. Agroforestree Database:a tree reference and selection guide version 4.0. World Agroforestry Centre, Kenya.

Patras, A., Brunton, N., O'Donnell, C.P. and Tiwari, B. 2010. Effect of thermal processing on anthocyanin stability in foods; mechanisms and kinetics of degradation. Trends in Food Science & Technology. 21 (1): 3–11.

Reyes, L.F. and Cisneros-Zevallos, L. 2007. Degradation kinetics and colour of anthocyanins in aqueous extracts of purple- and red-flesh potatoes (Solanum tuberosum L.). Food Chemistry. 100: 885-894.

Saric, G., Markovic, K., Vukicevic, D. and Lez, E. 2013. Changes of Antioxidant Activity in Honey after Heat Treatment. Czech Journal of Food Science. Czech Journal of Food Science. 31 (6): 601-606.

Shantha, T. R., Reddy, M. P., Rao, V. R., Bharthi, V., Kumar, R.K and Venkateswarlu, G. 2015. Pharmacognostic and Preliminary Phytochemical Studies on the Flowers of Kovidra: *Bauhinia purpurea* L. Journal of Drug Research in Ayurvedic Science 1(1): 47-60.

Shreedhara, C.S., Vaidya, V.P., Vagdevi, H.M., Latha, K.P., Muralikrishna, K.S. and Krupanidhi, A.M. 2009. Screening of *Bauhinia purpurea* Linn. for analgesic and anti-inflammatory activities. Indian Journal of Pharmacology. 41 (2): 75-79.

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.

Wang, W.D. and Xu, S.Y. 2007. Degradation kinetics of anthocyanins in blackberry juice and concentrate. Journal of Food Engineering. 82: 271-275.

Wesche-Ebeling, P. and Montgomery, M.W. 1990. Strawberry polyphenol oxidase: its role in anthocyanin degradation. Journal of Food Science. 55: 731-734.