Colour Behaviour and Stability of Anthocyanin in the Blue Flower of *Stachytarpheta jamaicensis* and *Hydrangea macrophylla*

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**Abstract.** The colour behaviour at pH 1-11 and the stability at pH 3 of anthocyanins extracted from the blue flower of *Stachytarpheta jamaicensis* (SJ) and *Hydrangea macrophylla* (HM) has been studied. The aqueous extract of both sources exhibited a wide range of colour from red to yellowish green. The colour intensity, total monomeric anthocyanin, and total phenolic content of SJ were higher than those of HM. Their colour was almost disappeared at pH 4-5 and rapidly degraded at pH 7-8, that made them a poor candidate for the colourant at the pH range. The stability of both extracts was tested at pH 3 in a 30°C incubator for 10 days. There were no significant differences in the degradation rate of colour intensity and the total phenolic compound of both extracts. The monomeric anthocyanin in HM degraded significantly faster than that in SJ. Overall, the SJ performed better than HM.

1. Introduction

Anthocyanins are the most interesting water-soluble plant-based natural colourant due to their wide range of colour including red, magenta, purple and blue [1]. The colour of an anthocyanin depends on many factors, especially pH. It is already well-known that, in general, anthocyanins are red at pH < 3 because of the presence of flavylum cation (AH⁺) as predominant species. They are almost lost the colour at pH 4-5 due to the conversion of AH⁺ to colourless hemiketal (B) through hydration. At the higher pH, they are purple or blue because of the existence of neutral and anionic quinonoidal base (A and A⁻, respectively) as the main species. Interestingly, certain anthocyanins are blue at pH 4, as found in butterfly pea flower (*Clitoria ternatea*) extract [1]. The anthocyanins in this flower are recognised as one of the most stable anthocyanins [2].

Other than *C. ternatea*, there are several plants grown in Indonesia that reproduce blue flowers, such as Pecut Kuda (*Stachytarpheta jamaicensis*) and Kembang Bokor (*Hydrangea macrophylla*), are naturally blue. *S. jamaicensis* is a weedy herbaceous plant that has a smooth and dark green stem, that turns woody towards the base of the stem [3]. *S. jamaicensis* is an underutilised plant that widely found in acclimatised tropics like in Indonesia and Malaysia. To our knowledge, there is no study related to the anthocyanin content of the blue flower of this plant. H. *macrophylla* is a landscape shrub that consists of many cultivars. The colour of the flower of this plant varies, depend on the pH. The main anthocyanin in the flower of H. *macrophylla* is delphinidin-3-glucoside [4], which classified as unacylated anthocyanin. The unacylated anthocyanins are very unstable in a low acidic and neutral condition. However, delphinidin-3-glucoside might configure a complex called metalloanthocyanin...
with Al$^{3+}$ to provide stable blue colour [4, 5]. The configuration explained why the flower of H. macrophylla is blue in acidic soil. Only in the acidic soil the Al$^{3+}$ presents and be transported through the roots to the flowers of the plant, as presented somewhere else [4]. Furthermore, to find the potential source of the anthocyanin-base blue colourant, it is important to study the colour characteristics and the stability of an anthocyanin extracted from the flower of S. jamaicensis and H. macrophylla.

2. Materials and Methods

2.1. Materials

The flowers of S. jamaicensis (SJ) were obtained from plant wildly grow at Tabalong district, South Kalimantan, Indonesia. The flowers of H. macrophylla (HM) were obtained from Tangerang, Banten, Indonesia. The flowers were dried for 24 hrs at 45°C, pulverised and sieved through 250 μm screen, packed in a tight container and kept in a freezer until used. The deionised water was obtained from a local market (Amidis®). The Folin-Ciocalteu reagent, hydrochloric acid (HCl), sodium hydroxide (NaOH), acetic acid, gallic acid, sodium carbonate, ethanol 96%, sodium acetate, monopotassium phosphate and dipotassium phosphate were purchased from Merck kGaA, Darmstadt, Germany. All chemical used were analytical grade and directly used without further purification.

2.2. Extract Preparation and Colour Quality Determination

The extraction of anthocyanins from the flowers was arranged by following the method described in the previous research [1]. The extracts were adjusted to pH 1 to 11 by 0.1 M HCl or 0.1 M NaOH depend on the targeted pH with different dilution factor depend on their initial colour intensity. The light spectra of SJ and HM extracts was recorded at a visible region (400-700 nm) by the UV-Vis Spectrophotometer (Genesys 10uv Thermo Electron Corporation, USA). The spectroscopic data were used to determine the wavelength giving maximum absorption ($\lambda_{\text{max}}$), colour intensity (CI), and browning index (BI).

The CI was determined as ($A_{\lambda_{\text{max}}} - A_{700}$) x DF, while the BI as ($A_{430} - A_{700}$)/ ($A_{\lambda_{\text{max}}} - A_{700}$) [1]. $A_{\lambda_{\text{max}}}$ was an absorbance at a wavelength with maximum absorbance, and DF was a dilution factor. $A_{430}$ was an absorbance at 430 nm and $A_{700}$ was an absorbance at 700 nm.

2.3. Stability Analysis

The stability test was exclusively studied at pH 3. Both extracts were packed in a 50-ml dark glass vial and stored in an incubator at 30°C without light exposure for 10 days. Each extract consisted of three replications. The CI, total monomeric anthocyanin (TA) and total phenolic content (TP) of the samples were determined every day.

The TA was determined as cyanidin-3-glycoside using the pH differential method [6]. A 0.2 ml sample was diluted with 1.8 ml buffer solution pH 1.0 and 4.5. The absorbance of both samples measured at 510 and 700 nm. The difference between absorbancies were calculated as the following:

$$A = (A_{510} - A_{700})_{\text{pH 1.0}} - (A_{510} - A_{700})_{\text{pH 4.5}}$$  \hspace{1cm} (1)

The TA (cyanidin-3-glucoside equivalents in mg/L) was obtained by:

$$\left( A \times \text{MW} \times \text{DF} \times 1000 \right) / (\varepsilon \times l)$$  \hspace{1cm} (2)

MW is molecular weight of cyanidin-3-glucoside (449.2 g/mol), DF is a dilution factor, $\varepsilon$ is molar absorptivity of cyanidin-3-glucoside which is equal to 26 900, and l is the cuvette width. The TP was measured by the Folin-Ciocalteu (FC) method with gallic acid as the standard [6]. A 0.2 ml sample was reacted with 0.8 ml 20% sodium carbonate and 1 ml FC reagent. After an hour, the absorbance...
was observed at 765 nm. The calibration curve was constructed by the reaction of 0.2 ml gallic acid at several levels of concentration with 0.8 ml of 20% sodium carbonate and 1 ml FC reagent. The total phenolic was determined as gallic acid equivalent (GAE) through the following formula:

\[ \text{TP (mg/L GAE)} = \frac{\text{Abs}}{m} \]  

Abs was the absorbance (A) and m was the slope of the gallic acid standard curve.

2.4. Degradation Kinetics
The degradation kinetics of CI, TA, and TP were evaluated using the first order reaction (Eq. 4), then the \( t_{1/2} \) was determined.

\[ C = C_o \cdot e^{-kt} \]  

\[ t_{1/2} = \frac{\ln 2}{k} \]  

\( C \) was the final concentration and \( C_o \) was the initial concentration, \( k \) was the constant of reaction rate (day\(^{-1}\)).

2.5. Statistical Analyses
The statistical analyses involved were Pearson’s correlation test and regression slope test. The analyses were conducted with the help of Microsoft Excel software.

3. Results and discussion

3.1. Colour Behaviour and Quality
Both SJ and HM extracts exhibited a wide range of hue at pH 1 to 11 as depicted at figure 1. This behaviour promotes them as a potential pH indicator. Two important colour quality of an anthocyanin-source extract that commonly evaluated are colour intensity (CI) and browning index (BI) [7]. The CI determines the intensity of coloured species which are red flavylium cation \( \text{AH}^+ \), purple quinonoidal base \( A \) and blue anionic quinonoidal base \( A^- \). The increase of browning index indicates a decrease in the intensity of coloured species and the increase of pale-yellow species chalcone \( C \) \( (A_{420}) \). The measurement of BI is commonly used to monitor the colour degradation [1,7].

As seen in figure 2, SJ and HM extracts performed relatively similar colour quality. The highest CI appeared at pH 1 when the only species existed was \( \text{AH}^+ \). At this pH, the CI of SJ was about two times of that of HM. Thus, the total anthocyanin content of SJ extract was approximately twice as high as that of HM extract. Both extracts were almost colourless at pH 4-5 due to the presence of hemiketal \( B \) as the predominant species. This was typical for the non-polyacylated anthocyanin as also observed in the extract \( \text{Melastoma malabathricum} \), \( \text{Rhodommyrtus tomentosa} \), \( \text{Vitex pinata} \), and \( \text{Clidemia hirta} \) fruits and \( \text{Tibouchina semidecandra} \) flower [7]. This result was in accordance with the prior work that the main anthocyanin in HM extract was an unacylated anthocyanin (delphinidin 3-glucoside) [4]. The very low intensity at pH 4-5 also gave an early sign that the major anthocyanin in SJ extract was a non-polyacylated anthocyanin.

The relatively high CI of both extracts appeared at pH ≤ 3 and pH 7-8. Meanwhile, The BI was relatively low at pH ≤ 6 and dramatically increased as the pH rise to 7. Accordingly, both extracts are exclusively potential as a food colourant at a high acidic condition. Based on the CI, SJ extract was better than HM extract.
3.2. Stability Study

The stability of the extracts was studied by monitoring the decrease of their CI, total monomeric anthocyanin content (TA), and total phenolic content (TP) during storage at 30°C. Compare to HM extract, SJ extract was higher both in TP and TA. Total phenolic of SJ and HM extracts were 801.23 and 276.32 mg/l, respectively. Total monomeric anthocyanin in SJ was about 2.3 times that in HM extract.
HM extracts (100.32 mg/l vs 44.21 mg/l). Initially, based on the CI, there was three pH level chosen for the stability study: 3, 7, and 8. Unexpectedly, the colour of both extracts at pH 7 and 8 was very unstable and rapidly fade within hours. Prior to the research, we thought that the anthocyanin in HM extract would be in a complex with Al$^{3+}$ to form a metalloanthocyanin. Hence, its stability at a neutral and low basic condition was expected to relatively high as performed by the other metalloanthocyanin like commelinin, protocyanin, protodelphin, and cyanosalvanin [8]. We also expected that as its colour was naturally blue, the SJ extract would relatively stable at a relatively high pH condition. However, the finding indicated that the anthocyanin in both extracts was in a monomeric state. In this state, the anthocyanin rapidly loses its colour at low acidic and neutral conditions [1]. Therefore, the probability of both extracts to be a blue food colourant in such condition was less likely.

As the consequence of the result above, the stability of both extracts was exclusively studied at pH 3. As a note, at pH 3, the CI of HM was only 42.3% of that at pH 1. It implied that more than 50% of AH$^+$ in the extract was already converted to B. At the same pH, the SJ extract kept almost 90% of the AH$^+$. Summarily, the SJ extract performed much better initial colour intensity than the HM extract at pH 3.

Table 1 summarises the first order degradation rate ($k$) and $t_{1/2}$ for the CI, TA, and PC of both extracts. The correlation test showed that there was a strong positive correlation among three parameters with the Pearson correlation coefficient $\geq 0.86$. In another word, the total monomeric anthocyanin and phenolic compound decreased as the decrease of the colour intensity.

| Stability Parameter | $k$ (day$^{-1}$) | $t_{1/2}$ (day) | p-value of slope test |
|--------------------|-----------------|----------------|----------------------|
|                    | SJ  | HM  | SJ  | HM  |                |
| CI                 | 0.021 | 0.020 | 32.76 | 34.35 | 0.76 |
| TA                 | 0.017 | 0.028 | 41.85 | 24.86 | 0.01 |
| TP                 | 0.018 | 0.018 | 39.58 | 37.68 | 0.78 |

Anthocyanin degradation consists of reversible colour degradation and irreversible chemical degradation [9]. An anthocyanin could undergo the colour fading without the chemical degradation. In this stage, the colour was dramatically decrease and the anthocyanin remains stable. As the result, the k of CI is much higher than the k of TA. In contrary, both extracts showed a small gap in the k of CI and TA. Rationally, the extracts lose their colour as they lose the anthocyanin structure. This was in alignment with the degradation mechanism of anthocyanin at a low pH that initiated by the chemical degradation of anthocyanin to its aglycon and followed by the colour degradation [10]. The aglycon was unstable and degraded further to 4-hydroxybenzoic acid and benzaldehyde derivative [10].

The regression slope test was applied to compare the k of all stability parameter between SJ and HM extracts. As seen in table 1, the colour and total phenolic stability of both extracts were statistically similar. Inversely, the monomeric anthocyanin in HM extract degraded significantly faster than in SJ extract. Based on the anthocyanin stability, the SJ was considered as better anthocyanin-source plant than HM.

4. Conclusion
The colour behaviour of the extract of *Stachytdarpheta jamaicensis* and *Hydrangea macrophylla* flower was relatively similar. Their best performance as a colourant appeared at the high acidic condition when the predominant species was the red flavylium cation. The half-life of their colour at pH 3 was
more than 30 days. Based on the initial colour intensity, total phenolic content and anthocyanin stability the \textit{S. jamaicensis} was better than \textit{H. macrophylla} extract. Both sources performed poorly at pH > 3. Their colour almost disappeared at pH 4-5 and degraded rapidly at pH 7-8. The improvement through a metal complexation and or intermolecular copigmentation might be considered to increase their chance to be a blue colourant that is highly stable at the low acidic and neutral condition.

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