

Color Properties and Stabilizing Effect of Metal Ion on Anthocyanin from *Buni* (*Antidesma bunius*) Fruit

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ABSTRACT

The objectives of this research were to evaluate the possibility of metal complexation in generating blue color, and the stability of the anthocyanin from *Buni* (*Antidesma bunius*) fruit extract. Four different metals ions (Ca^{2+} , Mg^{3+} , Al^{3+} , and Fe^{3+}) have tested at three concentrations level (0 ml, 0.01 ml, and 0.02 ml of 0.1 N solution per ml extract). The divalent Ca^{2+} and Mg^{2+} ions made an impact minimally to the bathochromic and hyperchromic shift at pH 3-8. The trivalent Fe^{3+} successfully generated a bluer color at all pH, while the Al^{3+} did not work only at pH 3. Stability test at pH 6-8 showed that the best stability occurred at pH 6. However, the metal complexation samples performed lower stability than the control samples.

Keywords: anthocyanin, bathochromic shift, co-pigmentation, hyperchromic shift, metal ion

I. INTRODUCTION

Anthocyanin recognized as one of the most potent natural colorants because of its solubility in water, a wide range of color, relatively low toxicity, and functional benefit [1]. The major constraint of the application of anthocyanin in food products is its low stability, especially at a low acidic and neutral condition [1].

There are several methods to increase the anthocyanin stability, including co-pigmentation, self-association, and metal complexation. Tin (Sn), copper (Cu), iron (Fe), aluminum (Al), magnesium (Mg), and potassium (K) are amongst the most common metal used in the complexation. Only anthocyanins that have more than one free hydroxyl group in the B-ring, such as cyanidin, delphinidin, and petunidin based anthocyanins, are able to form a complex with the metal. The complexation may increase the color intensity (hyperchromic shift) and change the red color into bluer (bathochromic shift) and more stable color [2] as reported to be occurred in delphinidin 3-glucoside [3]

Buni (*Antidesma bunius*) fruit is a wild plant grown in several mountainous areas in Indonesia such as Java, Bali, and Borneo Island. The fruit reported rich in flavonoids, including anthocyanins [2]. The primary anthocyanins in Buni fruit are cyanidin-O-glucoside and malvidin 3,5-diglucoside [2]. In spite of numerous and well-documented studies on the co-pigmentation of anthocyanin, information is lacking on the metal complexation effect of

the fruit extract with four different metal ion such as Ca^{2+} , Mg^{2+} , Al^{3+} , and Fe^{3+} in the development of blue color.

II. METHODS

Materials

This Buni fruits (Bekasi, West Java, Indonesia) washed, steam-blanching for six minutes, freeze-dried for 72 hours, powdered and sieved through 250 μm screen. The powder packed in a tight container and kept in a freezer until used. The chemical used were Folin-Ciocalteu reagent, sodium carbonate, sodium acetate, monobasic potassium phosphate, dibasic potassium phosphate, ferric (III) chloride, aluminum (III) chloride, calcium (II) chloride, magnesium(II) (Merck, Darmstadt, Germany), thanol 96%, HCl 36%, DPPH (2,2-diphenyl-1-picrylhydrazyl) and glacial acetic Acid 99.9% (Sigma-Aldrich, St. Louis, MO). All chemicals were analytical grade and used without further purification. Buffer solution pH 3 – 6 was made from sodium acetate and acetic acid. Buffer solution pH 7 – 8 was made from monobasic potassium phosphate and dibasic potassium phosphate.

Extraction

One gram of *buni* powder extracted in 40 ml of 0.1 M HCl with no light and continuous shaking for 30 minutes, followed by centrifugation at 8000 rpm for 4 minutes. The supernatant and liquid separated using Whatman filter paper No 1.

The Addition of Metal Ion

The extract diluted with buffer solution pH 3 – 8 with a dilution factor (DF) of 10. The 0.1 N metal ion solution was added to each diluted extract at three levels of ratio: 0, 0.01, and 0.02 ml per ml extract. All samples were equilibrated for around 1 hour at room temperature. The absorption spectra of samples were monitored using a UV-Vis spectrophotometer (Genesys 10uv Thermo Electron Corporation, USA). A bathochromic shift ($\Delta\lambda_{\max}$) was detected as a shift of wavelength expressed in nm and Hyperchromic effect (%A) as an increase in the absorbance value at $\lambda_{\text{vis-max}}$. Hyperchromic effect (%A) were calculated with the equation:

$$\%A = \frac{A - A_0}{A_0} \times 100\% \dots \dots \dots (1)$$

A_0 was the absorbance at $\lambda_{\text{vis-max}}$ of sample without metal ions, A was the absorbance at $\lambda_{\text{vis-max}}$ of sample with metal ions, and $\lambda_{\text{vis-max}}$ was the wavelength at visible region that give the highest absorbance.

Color Intensity Analysis

The color intensity (CI) measured as:

$$CI = (A_{\lambda_{\text{vis-max}}} - A_{700}) \times DF [4] \dots \dots \dots (2)$$

$A_{\lambda_{\text{vis-max}}}$ was the absorbance at $\lambda_{\text{vis-max}}$, A_{700} was the absorbance at 700 nm, and DF was the dilution factor.

Degradation Kinetics

Color lost assumed to follow first-order degradation kinetics, and linear regression analysis was used to determine acceptability of the treatments. The k-values and t (1/2) values for the first-order reactions were determined using the following equation:

$$n \frac{A_t}{A_0} = -k \times t \dots \dots \dots (3)$$

$$t_{1/2} = -\ln 0.5 \times k^{-1} \dots \dots \dots (4)$$

III. RESULTS AND DISCUSSION

The color and visible light absorbance of buni extract at pH 3-8 are depicted in Figure 1. The λ_{\max} of the extract at pH 3-8 was 523, 528, 534, 556, 576, and 591 nm, respectively. The highest intensity of the extract appeared at pH 8, while the lowest intensity occurred at pH 5. Based on the color intensity, the buni extract might be considered as a potential coloring agent at pH 3, 7, and 8. Besides color intensity, the browning index (the ratio between the absorbance at 420 nm and the absorbance at λ_{\max}) is also important to determine the quality of the extract as a coloring agent [1]. The browning index of the extract at pH 3, 7, and 8 was 0.34, 0.73, and 0.58. The low browning index is favorable. Therefore, the highest quality of buni extract as a coloring agent appeared at pH 3.

The Effect of Metal Addition

Four metal ions – two divalent ions and two trivalent ions – were investigated in this study. Visually, Fe^{3+} was the most affecting ion to the color shift of buni extract at all pH studied (Figure 2). The spectrophotometric observation showed that the divalent ions (Ca^{2+} and Mg^{2+}) gave no impact or tended to slightly decrease the color of the extract at all pH studied. Meanwhile, the trivalent ions (Al^{3+} and Fe^{3+}) showed the color shift at all pH studied, except Al^{3+} at pH 3.

There are two types of spectroscopic shift: vertical and horizontal shift. The vertical shift is related to the chroma or saturation (intensity). The hyperchromic shift (upward shift) indicate the increase of color intensity, while the hypochromic shift (downward shift) indicate the decrease of color intensity. The horizontal shift is related to the hue. The bathochromic shift (shift to the right or to the longer wavelength) indicates the change of hue to bluer, while the hypsochromic shift (shift to shorter wavelength) indicates the change to redder hue.

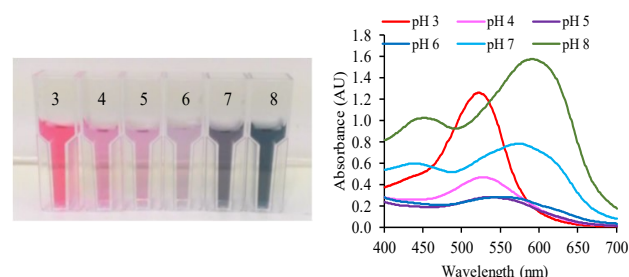


Figure 1. color and light absorbance of *buni* extract at pH 3 to 8

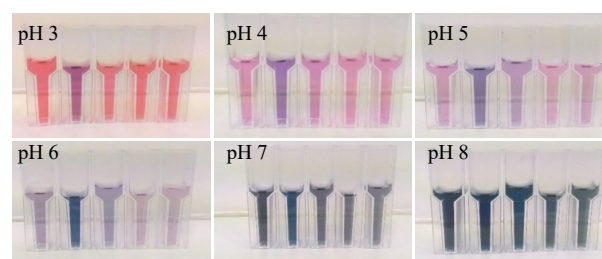


Figure 2. Color changes of anthocyanin at pH (3-8) after metal complexation with (from left to right): control, Fe^{3+} , Al^{3+} , Mg^{2+} , and Ca^{2+}

The higher amount of trivalent ions added gave higher both hyperchromic and bathochromic shift. The range of hyperchromic shift was 1.82 to 330.20% of the initial intensity. The range of bathochromic shift was 2 to 29 nm. The Fe^{3+} caused a higher hyperchromic shift than Al^{3+} in the extract at all pH studied.

The highest hyperchromic shift by Fe^{3+} was in the extract at pH 6 followed by at pH 5, 4, 7, and 8. Meanwhile, the order of the hyperchromic shift by Al^{3+} from the highest to the lowest was at pH 6, 5, 8, and 3. It was assumed due to the anthocyanin species at pH 6, which was neutral quinoidal base already converted to the anionic quinoidal base by the presence of metal ion.

Since the neutral quinoidal base has no charged in flavylium ring and less acidic environment at pH 6, so it was easier for metal with positive charge coordinates with anthocyanin and gave an intense blue color.

The greatest bathochromic shift occurred in the extract at pH 6. The Fe^{3+} and Al^{3+} bathochromically shifted the color of 26 and 18 nm, respectively. The shift was likely due to the electron configuration of each metal. Fe^{3+} ion was thought in a high spin configuration, which having one electron in each of the d orbitals. Whereas Al^{3+} only had one electron in the P orbitals. For divalent metal ions were greater than divalent, it was suggested due to the ion-dipole interaction, which Mg^{2+} and Ca had lower positive charged, causing a minimum shift of bathochromic [5].

The Stability Test

The color stability of anthocyanin complexed by 0.2 ml 0.1 N FeCl_3 /ml extract (ACN-Fe^{3+}) was observed at pH 6 to 8 both at room and refrigerator temperature. In accordance with the previous research that the color stability of anthocyanin decrease as the pH increase [6], the color of *buni* extract at pH 6 was much more stable than at pH 7 and 8 (Table 1). The $t_{1/2}$ of the extract at pH 7 and 8 were shorter than two days. Meanwhile, the color stability of *buni* extract at pH 6 was 8.48 to 27.18 days.

As seen in Table 1, the anthocyanin complexation with iron was proven to improve the color stability. The same effect of metal complexation was also reported to be occurred in different berry products [6]. In contrast, the addition of Fe^{3+} tended to decrease the color stability of *buni* extract at pH 6.

Figure 1 depicted that *buni* extract demonstrated the lowest intensity at pH 5 and 6. The pale color indicated that the predominant species of anthocyanin at respective pH, while the remaining red color might contributed by the polymeric anthocyanin that relatively stable. Therefore, the color stability of the extract was relatively high.

The hydration of red flavylium cation to colorless hemiketal is a reversible reaction. The addition of Fe^{3+} converted the simple anthocyanin to an anthocyanin-iron complex and reverse the hemiketal to flavylium cation. The flavylium cation deprotonated to quinoidal base to form the blue color. However, the low stability indicated that the complex was relatively weak and tended to destruct during storage. Further research is needed (i) to reveal the mechanism of the complexation, (ii) to understand how the complex destructed, and (iii) to improve the complex stability.

IV. CONCLUSION

As a summary, the finding from present study provides detailed color properties information including the hyperchromic shift and bathochromic effect of the copigmentation between anthocyanin and metal ions were obtained. The specific metal ion were found to impact the properties of the observed colors of anthocyanin. Divalent Mg^{2+} and Ca^{2+} were impact minimally, inducing very small or did not impact to bathochromic shift and hyperchromic effect. The trivalent metal Fe^{3+} and Al^{3+} induced significantly change on the color spectral characteristic of anthocyanin.

Stability studies showed that iron complexation increased the stability of *buni* extract at pH 7 and 8, but tended to decrease the stability at pH 6.

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