Co-pigmentation of purple sweet potatos (Ipomoea batatas L) anthocyanin extract using green tea extract

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Abstract. Copigmentation is one of the methods to stabilize and enhance the anthocyanin color. Green tea has the phenolic compounds (one of them is catechin) that potentially serves as co-pigment. In this study co-pigmentation of purple sweet potato (Ipomoea batatas L) anthocyanin extract with green tea extract were investigated. The anthocyanin was extracted with water at pH 2. Green tea extract was made by maceration in hot water by varying time. The co-pigmentation was conducted by varying ratio of anthocyanin extract with green tea extract (1:1; 1:2; 1:3; 1:4; and 1:5). The results showed that the total anthocyanin in anthocyanin extract from purple sweet potato was 1700 ppm, the total polyphenol in green tea extract was 10014 ppm. Green tea extract can be used as co-pigment for anthocyanin from purple sweet potato by evaluating the bathochromic effect (maximum wavelength shift, \(\Delta \lambda_{\text{max}}\)) and hyperchromic effect (the increase of absorbance, \(\Delta A_{\text{max}}\)). Green tea extract showed the best co-pigmentation in the ratio (1:1) while the bathochromic effect (\(\Delta \lambda_{\text{max}}\)) was 4 nm and hyperchromic effect (\(\Delta A_{\text{max}}\)) was 0.548.

1. Introduction
The use of natural color in foods and beverages has increased as substitutes for their synthetic colorants. This is caused the increasing awareness of the environmental hazards and the potential side-effect impacts of synthetic food coloring [1]. Food colorants from plant tissue are widely developed, especially from some edible sources, one of them are anthocyanins. Anthocyanins pigments are water-soluble, widely distributed in the plant. The molecules of anthocyanin are flavonoids group, play a role for the color of red, purple, and blue. Fruits and vegetables were source of anthocyanin extracts can be used as food colorants to substitute the synthetic color. Anthocyanins also showed some biological functions, like antioxidant, anti-carcinogen activities, anti-inflammatory, hepatoprotection capacity and the ability to enhance memory [2][3]. Anthocyanins can also increase the processed foods nutritional value by preventing lipids and proteins oxidation in the food products [4]. Therefore, increasing the application use of anthocyanin in food and beverages industry is very important because of their potential health benefits besides attractive colors.

Anthocyanins, like most natural pigments, are unstable and susceptible to degradation easily. The stability of anthocyanin color is affected by pH, solvents, temperature, concentration, structures of anthocyanins, oxygen, light, enzymes, and other substances attached to them. The anthocyanin color stability can be improved by co-pigmentation. Copigmentation is a phenomenon where natural dyes and colorless organic compounds or metal ions form molecular or complex associations. In co-
pigmentation, the anthocyanin molecule reacts with co-pigment directly or through weak interactions, producing an enhanced and stabilized color. Copigments are colorless, but when added to the anthocyanin solution, they increase the color intensity and the stability of the solution.

Copigment compounds generally are flavonoids, polyphenols, alkaloids, organic acids, and amino acids. Flavonoids are well-known co-pigments among which flavons, flavonols, flavonons, and flavanols have been vastly studied. Polyphenols are compounds that are naturally found in plants, contain many phenol groups that can function as antioxidants and are one type of co-pigment that can be used to improve the stability of natural dyes. Green tea has the rich phenolic compound that potentially serves as co-pigment. The major constituents in tea polyphenols are catechins with flavan3-ols structures and their polymerized products. The polyphenols content in green tea can reach up to 30% by weight [5]. The high content of polyphenols in green tea allows it to become a co-pigment for anthocyanins. The current study aims to co-pigmentation anthocyanin extract of purple sweet potato with the green tea extract. Copigmentation phenomena are observed as a bathochromic shift (maximum wavelength shift,) and hyperchromic effect (the increase of absorbance).

2. Materials and Methods

2.1. Materials
The materials for this research are the purple sweet potato obtained from Bogor, green tea and citric acid (food grade) from local market, folin ciocalteu reagent, sodium carbonate (20%), and gallic acid from Merck.

2.2. Methods

2.2.1. Anthocyanin extraction. The anthocyanin extraction was done based on previous work [6]. The washed and dried purple sweet potato are cut into small pieces, blanched at 70 ° C by adding water 1:2 (w/v) for 10 minutes, and then filtered. The filtrate was added citric acid until pH 2, then mix with the pulp and blended briefly, heated in 90 C for 10 minutes, and then filtered. The filtrate is centrifuged to precipitate the carbohydrate and concentrated using a rotary evaporator to obtained filtrate volume a half of initial volume. The total anthocyanin content was evaluated using the pH-differential method.

2.2.2. Green tea extraction. Extraction of green tea was done by maceration using water (1:2 w/v) at 80 ° C with varying of time (5 minutes, 10 minutes, 15 minutes, and 20 minutes). The extract is then filtered using carbon active and determined the total polyphenols using Folin Ciocalteu method [7]. A sample of diluted extract (20 µL) was added to distilled water (1.58 mL) in a test tube. After the addition of Folin-Ciocalteu reagent (100 µL) and saturated Na2CO3 20 % (300 µL), the solution was incubated at 40°C for 30 min. The absorbance of the samples was then measured at 765 nm. Total phenols of green tea extract were expressed as mg gallic acid (GAE). Green tea extract with the highest levels of polyphenols from the maceration process that will be used for purple sweet potato anthocyanin co-pigmentation.

2.3. Co-pigmentation.
Co-pigmentation was carried out by mixing purple sweet potato anthocyanin and green tea extract in varying volume ratio 1:1; 1:2; 1:3; 1:4; and 1:5. Absorption spectra of purple sweet potato anthocyanin solutions, with and without green tea extract were recorded using a UV-visible spectrophotometer, scanning the visible range from 450 nm to 600 nm. The maximum absorbance change (Amax) at varying wavelengths (λmax) presented the difference in the color intensity, and revealed the possibility of hyperchromic effect (ΔAmax) and bathochromic shift (Δλ), resulting from a co-pigmentation reaction. Optimal co-pigmentation products are stored at room temperature and the absorbance is measured daily and compared to antocyanins without co-pigment.
3. Results and Discussions

Polyphenols are chemical compounds with the basic structure of phenols, naturally present in plants and are useful as antioxidants. Polyphenol extraction from green tea was carried out by maceration using water as solvent at 80 °C in different times. The total polyphenol content (calculated as gallic acid) of each extract is shown in Figure 1. The highest total polyphenol content was obtained in maceration for 10 minutes with the total polyphenol was 10014.93 ppm (mg/L). Maceration with more than 10 minutes produced the darker extract and the lower total polyphenol. Prolonged heating caused the oxidation of polyphenols [8] so the total polyphenol was decreased after maceration for 10 minutes. The darker extract was also avoided because for applications as co-pigments can interfere with the intensity of color. The extract of green tea polyphenols which was carried out from maceration for 10 minutes be used as a co-pigment in this study.

![Figure 1. The polyphenol total of green tea extract in varying time of maceration](image)

Anthocyanins are the most abundant flavonoid constituent of red fruits and vegetables and are used as natural color which soluble in water [9]. These pigments are responsible for the red colors, blue, or purple. The use of anthocyanin in food and beverages have many benefits, besides their attractive colors, they have any function for healthy, like antioxidant, anticancer, and anti-inflammatory. The extraction of anthocyanin in purple sweet potatoes was carried out using water solvents by blanching method. Blanching is one of the most critical steps to increase the stability of purple sweet potato anthocyanins. Blanching can reduce the activity of peroxidase enzyme which makes the anthocyanin color become brownish. Analysis using an UV-vis spectrophotometer shows the optimum absorbance at wavelength 530 nm (Figure2) as in some previous research [10][11]. The concentrated anthocyanin extract contains a total anthocyanin level of 1700 mg/L (calculated as cyanidin glucoside)
Figure 2. Visible spectra of purple sweet potato anthocyanin extract

Anthocyanins have unstable properties and susceptible to degradation easily. The anthocyanins color stability can be improved by co-pigmentation. In co-pigmentation, an unstable dye molecule reacts with other natural components of the plant directly or through the interaction of covalent bond formation to produce a more stable color [11]. The covalent bond in anthocyanin molecules can occur with an organic acid, an aromatic acyl group, or a flavonoid [12]. Purple sweet potato anthocyanin co-pigmentation was carried out by adding green tea extract which was rich in polyphenol compounds. The co-pigmentation was conducted by varying ratio of anthocyanin extract with green tea extract (1:1; 1:2; 1:3; 1:4; and 1:5). Co-pigmentation of purple sweet potato anthocyanin has been done previously using organic acids (ferrulic acid and tannic acids) [13].

The result of the addition of green tea extract co-pigments at five concentration levels showed that the outcome of co-pigmentation is dependent on co-pigment concentration. Characteristics of co-pigmentation can be observed using the UV-vis spectrum to evaluate the presence of hyperchromic effects (increase in intensity/ΔA) or bathochromic shift (shift in wavelength toward visible light/Δλmax) [14]. Absorption spectra of anthocyanin and the co-pigmentation are shown in Figure 2.

Figure 3. The absorption spectra of anthocyanin (without and with copigment)
The phenomenon of bathochromic shift ($\Delta \lambda_{\text{max}}$) and hyperchromic shift ($\Delta A$) in each variation of co-pigmentation are shown in Table 1. Increasing green tea extract concentration decreases the absorbance of the outcome of co-pigmentation. The effect of bathochromic occurs in all co-pigmentation process with $\Delta \lambda_{\text{max}}$ is 4 nm. The effect of hyperchromic happens on co-pigmentation with ratio co-pigmentation are 1:1, 1:2, and 1:3. The co-pigmentation with ratio 1:1 give the greatest hyperchromic effect (0.548), so the best condition on co-pigmentation is in ratio 1:1. Co-pigmentation of purple sweet potato with green tea extract produces highly pigmented new anthocyanin catechin complexes linked by CH$_3$CH bridges [15]. These complexes linked results more stable color in anthocyanin.

Table 1. Optimization of copigmentation purple sweet potato anthocyanin using green tea extract

| No. | Anthocyanin : green tea extract (volume) | $\lambda_{\text{max}}$ (nm) | $\Delta \lambda_{\text{max}}$ | Absorbans ($A$) | $\Delta A$ |
|-----|--------------------------------------|----------------|----------------|----------------|---------|
| 1.  | Anthocyanin                          | 530           | -              | 0.872          | -       |
| 2.  | 1:1                                  | 534           | 4              | 1.420          | 0.548   |
| 3.  | 1:2                                  | 534           | 4              | 0.952          | 0.080   |
| 4.  | 1:3                                  | 534           | 4              | 0.898          | 0.026   |
| 5.  | 1:4                                  | 534           | 4              | 0.695          | -0.177  |
| 6.  | 1:5                                  | 534           | 4              | 0.630          | -0.242  |

Figure 4 showed the changes in absorbance of purple sweet potato anthocyanin and purple sweet potato anthocyanins with co-pigment (green tea extract) are stored at room temperature. Anthocyanin was easily degraded during storage, causing anthocyanin color fading, which were indicated by the decreasing of absorbance. The absorbance decreasing of anthocyanins by the co-pigmentation with green tea extract was slower than anthocyanin without green tea extract. The co-pigmentation of green tea extract can prevent anthocyanin degradation during storage in room temperature.

Figure 4. Influence of time storage in room temperature on absorbance of anthocyanin with and without co-pigment (green tea extract)

4. Conclusion

Green tea extract showed the best co-pigmentation in ratio purple sweet potato anthocyanin and green tea extract (1:1) while the bathochromic effect ($\Delta \lambda_{\text{max}}$) was 4 nm and hyperchromic effect ($\Delta A_{\text{max}}$)
was 0.548. The co-pigmentation of green tea extract can prevent anthocyanin degradation during storage in room temperature.

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