

Effects of Temperature and Heating Time on Degradation and Antioxidant Activity of Anthocyanin from Roselle Petals (*Hibiscus sabdariffa* L.)

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Abstract

*The effects of temperature and heating time on the degradation and antioxidant activity of anthocyanin compounds from roselle petals (*Hibiscus sabdariffa* L.) have been evaluated from this research. The purpose of this study is to determine, the degradation and antioxidant activity of anthocyanin compounds on variations of temperature and heating time. In this study anthocyanin was extracted from roselle petals using ethanol solvent at 25°C. Then the anthocyanin extract obtained was dissolved into aqueous solution. The solution was then heated at 40°C, 60°C and 80°C for 60 minutes. The degradation of anthocyanin compounds was analyzed by calculating the total of anthocyanin contents, while the antioxidant activity was evaluated using the DPPH method. The results showed that Total Anthocyanin Content (TAC) from the extract solution before heating was 144.28 mg/L. Meanwhile the total value of anthocyanin in the 60 minutes after heating at 40°C, 60°C and 80°C are 108.88 mg/L, 88.84 mg/L and 63.78 mg/L respectively. Heating at 80°C decreases antioxidant activity by increasing the IC₅₀ value to 86.8 ppm higher than the conditions before heating. The increase of temperature and length of the heating process made the anthocyanin structure degraded. Degradation was indicated by the reduction of total anthocyanin content and the decrease of antioxidant activity.*

Keywords: Roselle; Anthocyanin; Stability; Degradation; Antioxidant

1. INTRODUCTION

Plants can be used as a source of natural pigments and safer food coloring, they have a low toxic effect. Among the compounds in plants that can be used as natural dyes are anthocyanin compounds [1]. Anthocyanins are compounds that give the red, blue and purple colors in plants [2]. One of the plants that contain anthocyanin compounds is rosella flower (*Hibiscus sabdariffa* L.). From previous study anthocyanin compounds have antioxidant activity [3,4]. Petals from rosella can be used as a substitute source for synthetic antibiotics [5].

Temperature, heating time and storing time will affect anthocyanin stability [6]. Food and beverage processing usually involve heating from 50°C until 150°C. Anthocyanin stability is not only function of final heating temperature but also

influenced by intrinsic properties of product and processing such as pH, temperature of storage, chemical structure, light, oxygen, enzyme and protein [7]. The structure of anthocyanin can be shown in three types depend on the color there are Cyanidin, Pelargonidin, Delphinidin. The types of Anthocyanin structure show in figure 1.

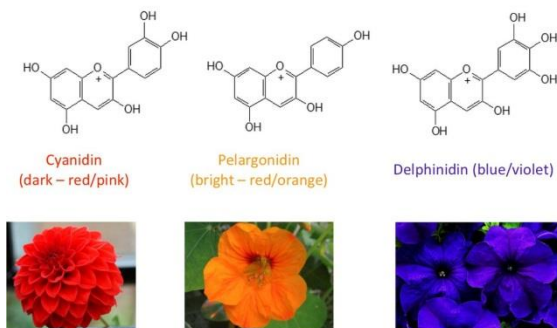


Figure 1. Types of Anthocyanin Structure

The three types of anthocyanin structures can be distinguished from the number of hydroxyl groups attached to the parent compound. The number of -OH groups contained in anthocyanins causes these compounds to have potential as antioxidants. Anthocyanin obtained by extracting roselle petals. Because the colour of Roselle petals extract were red so anthocyanin that were extracted known as Cyanidin-3-O-glucoside. Extraction process affecting the total of anthocyanin content. Based on study by Suzeryet, all., (2010), most optimum extraction process is maceration at 25°C [8].

Based on previous description, it should be known that anthocyanin easily changes on the structure. In this study, effect of solvent and heating (40°C, 60°C dan 80°C) on anthocyanin stability has been done. Solvent used were ethanol and distilled water. Stability of anthocyanin was determined by calculation of total anthocyanin content using pH differential method (Inggrid et, all., 2016) and antioxidant activity using DPPH method (Molyneux, 2004) [9,10]. Analysis was done using UV-Vis spectroscopy.

II. METHODS

This research was conducted in several stages. *Hibiscus sabdariffa* Linn was extracted in ethanol solvents. Extract was concentrated using *rotary vacuum evaporator* at 37°C and vacuum conditions 60 mbar. Effects of solvent on anthocyanin stability was done by calculating total anthocyanin content as cyanidin 3-glucosides using spectrophotometry UV-Vis.

II.1 Material and Tool

Materials used in this study are dried roselle petals purchased from MojosongoBoyolali Indonesia, ethanol, KCl, CH₃COONa, distilled water, methanol *pro analysis* (Merck, Germany), and HCl 37% (Merck, Germany), DPPH (2,2-diphenyl-1-picrylhydrazyl).

II.2 Preparation and Anthocyanin Extraction

Extraction of anthocyanin pigment was done using maceration at 25°C [8]. 100 mg of roselle petals powder macerated with 300 mL ethanol at 25°C for 24 hours. The produced filtrate then concentrated using *rotary vacuum evaporator* at 37°C and vacuum conditions 60 mbar.

II.3 Effects of Solvent on Total Anthocyanin Contents as Cyanidin 3-glucoside

Effects of solvent on anthocyanin stability was done by dissolving 0.025 mg anthocyanin crude extract on 10 mL distilled water and ethanol. Each solution was analyzed the maximum wavelength at 400-800 nm. Analysis of total anthocyanin content as cyanidin 3-glucosides was done using pH differential method.

A. Preparation of pH 1 Solution

1.490 g potassium chloride (KCl) was dissolved on distilled water using 100 mL volumetric flask. 25 mL KCl solution mixed with 67 mL of 0.2 N HCl. 0.2 N HCl was added into the solution until pH 1.0 ± 0.1 .

B. Preparation of pH 4.5 Solution

1.640 g sodium acetate was dissolved on distilled water using 100 mL volumetric flask. 0.2 N HCl N was added until pH 4.5 ± 0.1 .

C. Analysis of Total Anthocyanin Contents as Cyanidin 3-glucoside

0.050 g extracts was dissolved on 10 mL distilled water. This solution was split in half (5 mL each). Solution with pH 1.0 dan pH 4.5 was added into the extract solution. Absorbance of each solution was measured on 510 and 700 nm. Absorbance of dissolved samples (A) calculated using :

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

Total Anthocyanin content as cyanidin 3-glucoside of sample calculated using formula:

$$\text{Anthocyanin (mg/L)} = \frac{A \times MW \times DF \times 1000}{\epsilon \times L}$$

which:

ϵ = molar absorptivity molar cyanidin-3-glucoside (26900 L/ (mol.cm))

L = width of cuvette (1 cm)

MW = molecular weight of cyanidin -3- glucoside (449,2 g/mol)

DF = dilution factor

II.4 Effects of Heating on Total Anthocyanin Content

0.50 g extract was dissolved on 100 mL distilled water (5000 ppm). Each solution was heated at 40°C, 60°C, dan 80°C for 60 minutes. Total anthocyanin as cyanidin 3-glucoside was calculated before and each 10 minutes after heating. the solution was stored for antioxidant activity test.

II.5 Antioxidant Activity

Antioxidant activity was determined before and after heating process. Each extract solution was diluted with variation of 25, 50, 75, 100, 125 ppm. DPPH

solution was prepared by dissolving 20 mg DPPH powder on 100 mL methanol. 2 mL extract solution was mixed with 2 mL DPPH solutions [10]. Incubation was done for 30 minutes. The Absorbance of samples were measured by UV-Vis spectrophotometer at 517 nm using methanol as blank. Antioxidant activity (IC₅₀) was determined using calibration curve between concentration versus %inhibition. The %inhibition was calculated using formula:

$$\%inhibition = \frac{A_{blank} - A_{sample}}{A_{blank}} \times 100\%$$

III. RESULT AND DISCUSSION

The discovery of anthocyanin compounds in a given plant has a meaning that is very strategic and important because it can be used as material for antioxidant activity. In addition, researchers in Indonesia is currently being excited doing bioactive compounds that are detected and quantifies in plant material. *Hibiscus sabdariffa* is used as a target plant in our laboratory because it is a tropical plant and reportedly has a prospective anthocyanin compound for treatment of coloring agent and antioxidant.

III.1 Extraction of Anthocyanin

Extract yielded by extraction are 7.9107 g and 7.91% yield with dark red color. Extract used for analysis on different test parameter

III.2 Effects of Solvents on Total Anthocyanin Content as Cyanidin 3-O-glucoside

The maximum wavelength of the extract dissolved in ethanol is 543 nm, which has a tendency similar to the results obtained by Suzery, et al (2010) [8]. Whereas the maximum wavelength of distilled water obtained by distilled water is 519 nm. Both of these maximum wavelengths are uptake of anthocyanin. According to Wallace and Guisti (2013) the maximum wavelength of anthocyanin is in the range of 480 nm to 550 nm [11].

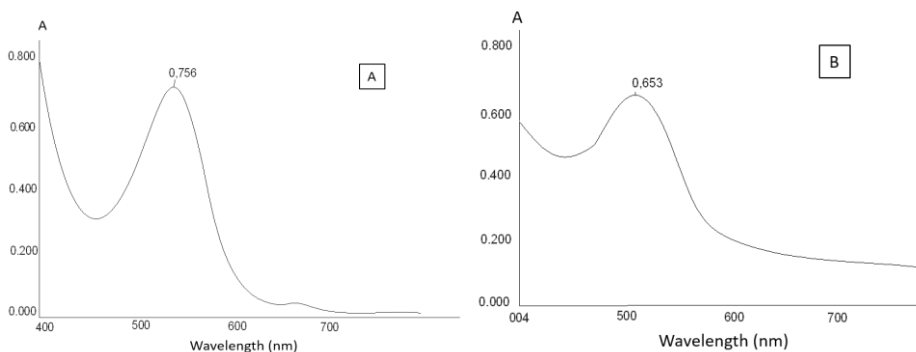


Figure 2. Spectrum of 400-800 nm wavelength (A) Ethanol (B) Distilled Water

The total content of anthocyanin as cyanidin 3-Glucoside obtained from distilled water was 144.28 mg/L, while ethanol was 171.66 mg/L. Ethanol solvent has a higher

total anthocyanin content as cyanidin 3-glucoside than distilled water. Ethanol and distilled water have the same properties, polar. The dielectric constant value of ethanol is 25.10 while water is 80.10 [12]. The Anthocyanin tend to have the same polarity properties as ethanol, causing the total content of anthocyanin produced in ethanol is higher than in water. Therefore, the anthocyanin compounds were more stable in ethanol solutions than in water. Anthocyanins have better stability in very acidic pH. This is evidenced by the anthocyanin dissolution test at pH 1 and pH 4.5. At pH 1, the color of the anthocyanins is dark red, but when it is at pH 4.5, the dark red color becomes faded. From the pH stability test, we also know that the main component in anthocyanins derived from roselle petals is cyanidin 3-glucoside. Cyanidin 3-glucoside containing in the Anthocyanin gives a red color.

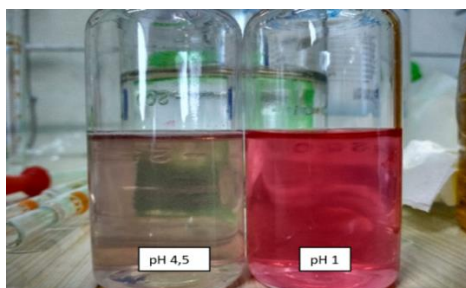


Figure 3.Anthocyanin colour depends on pH

III.3 Effects of Heating Process on Total Anthocyanin Contents

Total Anthocyanin Content (TAC) of extract solution before heating was 144,28 mg/L. Meanwhile, total anthocyanin content 60 minutes after the heating at 40°C was 108,88 mg/L; at 60°C was 88,84 mg/L; and at 80°C was 63,78 mg/L. Total anthocyanin content were changed after heating treatment due to the degradation of Anthocyanin structure [13]. Total anthocyanin contents at 80°C decreased up to approximately 80,5 mg/L. The total anthocyanin content in various temperature and time heating was showed on figure 4.

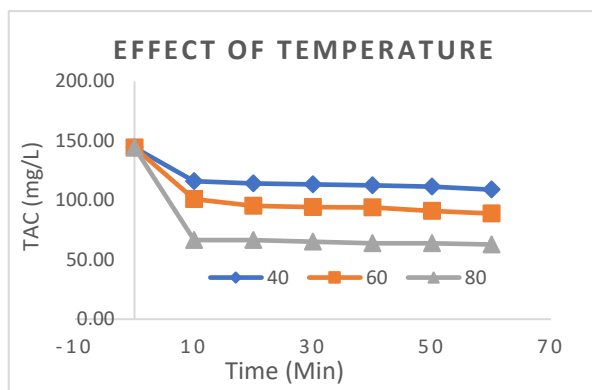


Figure 4. Anthocyanin concentration at temperature at 40°C, 60°C and 80°C

Total anthocyanin content at 40°C, 60°C, and 80°C tend to decrease until 60 minutes. As the rises of temperature, the more anthocyanin compounds were degraded [6]. Alteration of anthocyanin structure caused by increasing temperature happens with releasing of sugar molecules attached to anthocyanin structure, and transformation into aglycon named anthocyanidin. After releasing of sugar, there are cleavage process on the “C” ring. The result of cleavage bond separates the “A” ring and the “B” ring that induced formation of two new compounds there are Phloroglucinaldehyde and 4-hydroxybenzoic acid. The cleavage process of cyanidin lead changing color of cyanidin. The degradation of cyanidin-3-O-glucoside structure can be seen on Fig 5.

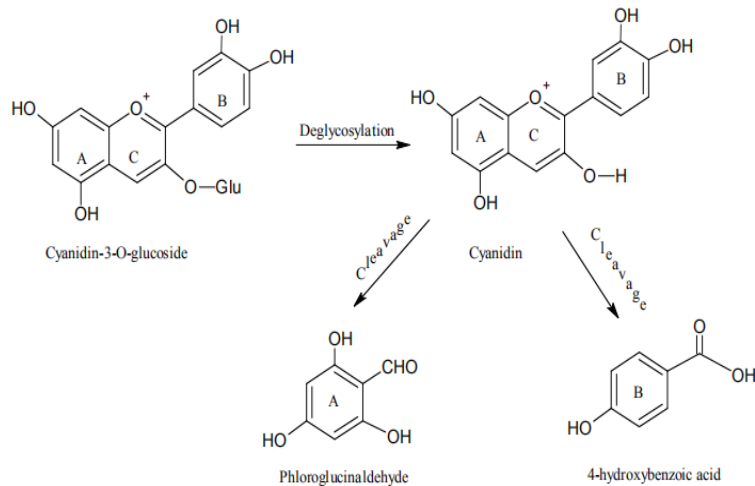


Figure 5. Mechanism of cyanidin-3-O-glucoside degradation

Table 1. Total anthocyanin content during heating process

Time (Min)	Total Anthocyanin Content		
	T:40°C	T:60°C	T:80°C
0	144,28	144,28	144,28
10	115,89	100,86	66,462
20	114,22	95,18	66,462
30	113,22	94,18	65,126
40	112,55	93,85	63,790
50	111,55	90,84	63,790
60	108.88	88,84	62,788

III.4 Analysis of Antioxidant Activity Using UV-Vis Spectroscopy

Analysis of antioxidant activity was done to quantify anthocyanin compounds after heating. From the analysis using UV-Vis Spectroscopy obtained data that heating treatment causes The IC₅₀ value of anthocyanin solution to increase. It means the degradation of Anthocyanin structure lead to decreasing of antioxidant activity. Therefore, Anthocyanin more stable at room temperature (without heating) than at heating temperature (40°C, 60°C, 80°C). The calibration curve of Anthocyanin antioxidant activity was showed on figure 6.

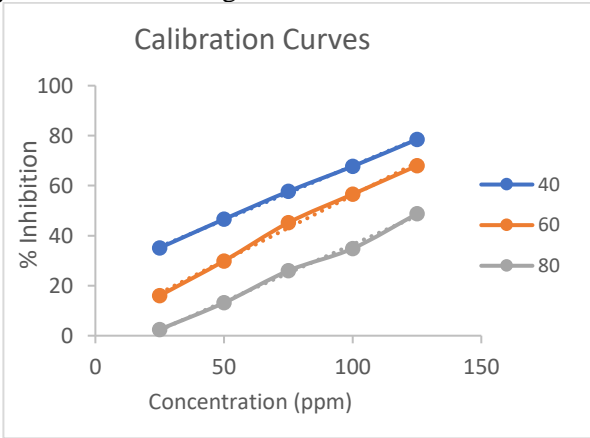


Figure 6. Calibration Curve at 40°C, 60°C, dan 80°C

Antioxidant activity of anthocyanin solution before heating has lower IC₅₀ value (42,81 ppm), while after heating treatment at 40°C, 60°C, and 80°C show increasing IC₅₀ value. Even the IC₅₀ at 80°C was increasing up to 202,75% than before heating or more than twice. It means Anthocyanin structure was very sensitive with heating treatment and unstable in higher temperature. These results showed that heating process reduce the antioxidant activity of anthocyanin very much. The IC₅₀ value of Anthocyanin showed in table 2.

Table 2. IC₅₀ Before and After Heating

Temperature	R ²	IC ₅₀ (ppm)
Before heating	0.984	42.81
40°C	0.994	57.64
60°C	0.995	88.15
80°C	0.996	129.61

IV. CONCLUSION

Extraction of anthocyanin compound from roselle petals was done using maceration. The types of Anthocyanin that extracted were Cyanidin-3-O-glucoside.

Ethanol solvent can maintain total anthocyanin content 27,33 mg/L higher than aqueous solvent. Aqueous contain high water activity that can interact through hydrogen bonding with -OH groups that attach in Anthocyanin structure, that interaction lead to degradation of anthocyanin. The temperature was very influential on the stability of anthocyanin structure which then have impacts on antioxidant activity of anthocyanin. As the higher temperature the more unstable anthocyanin structure because the degradation of Anthocyanin structure happened. When the Anthocyanin structure were degraded the anthocyanin ability as an antioxidant also reduce. From this research, it was obtained data that anthocyanins without heating had the smallest IC₅₀(42.81 ppm) of antioxidant activity. This is because no anthocyanin compounds were degraded because of heating treatment.

V. ACKNOWLEDGMENTS

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