Natural red dyes extraction on roselle petals

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Abstract. Roselle (Hibiscus sabdariffa L.) has a high quantity of anthocyanin pigment and is a good colorant. The anthocyanin pigment can be used as a natural colorant and antioxidant. An antioxidant is an organic compound that has the ability to inhibit free radical reactions in the human body. The objective of this research is to study the effect of pH and temperature on total anthocyanin and antioxidant activity in roselle extract, and to evaluate the effect of temperature and sunlight on the stability of the red color from roselle. Dried roselle petals were extracted with solid liquid extraction method using water as solvent. The variables in this study are temperature (5˚C, 30˚C, and 55˚C) and pH (2, 7, and 12). Total anthocyanin was analysed using the pH differential method. The antioxidant activities were determined using the DPPH method. The highest total anthocyanin in the roselle petals was 80.4 mg/L at a temperature of 5˚C and pH 2. The highest antioxidant activity and yield content in the roselle were 90.4% and 71.6% respectively, obtained at 55˚C and pH 2.

1. Introduction
Roselle is a plant of the malvaceae family and is native to the tropical areas such as Indonesia. This plant has good potential to be developed in Indonesia and is commonly found in the highlands in West Java. Along with agricultural technologic progress, anthocyanin compound is found in roselle petals and it also can serve as a source of antioxidants. Roselle is good for health because of its high antioxidant, and anthocyanin can be used as natural dye. Using natural dyes in food products is better and more safe compared to synthetic dyes [1]. Synthetic dyes give a negative impact to our body’s health, potentially being poisonous to our body’s metabolism. The most dominant anthocyanins of roselle petals are delphinidin-3-sambubioside and cyanidin-3-sambubioside [2]. These compounds are the ones that give roselle petals their red colour. Anthocyanin’s structure will be presented in Fig. 1. Anthocyanins are derivatives of the basic flavylium cation structure, which are highly reactive. The rate of anthocyanin destruction depends on many factors such as temperature, pH, ultraviolet light, and oxygen [2]. Anthocyanin dyes can be applied to food and drink products, such as jam, soft drinks, milk, and yoghurt [3].

Figure 1. The Structure of Anthocyanins [4]
In this experiment, water was used as a solvent because it produces a brilliant red color extract. Antioxidant activity of roselle extract is also pH dependent, the activity decreases as pH increases [2]. This experiment aimed to study the effect of pH and temperature on total anthocyanin and antioxidant activity of roselle petals, and also to determine the effect of temperature and sunlight on the stability of red color in roselle petals.

2. Materials and methods

Dried roselle petals were extracted using water as a solvent and extracted on F:S (1:15) with variation of temperature and pH conditions. These variations were analyzed to discover the highest total anthocyanin and antioxidant activity. The samples were analysed using spectrophotometry at anthocyanin wavelength (520 nm).

2.1. Sample preparation

Samples of roselle petals (*Hibiscus sabdariffa* L.) were taken from North of Bandung, Indonesia. The roselle petals were dried at 45°C for 24 hours to maintain moisture content below 10% before being subjected to anthocyanin extraction.

2.2. Sample extraction

The extraction for each sample was performed in duplicate. Briefly, 20 grams of dried roselle petals (-50+60 mesh) was transferred to 1 L batch extractor to which buffer (pH 2, pH 7, and pH 12) was added, and the mixture was mixed at 200 rpm, while temperature condition used is 5°C, 30°C, and 55°C. The mixture was stirred for 5 hours and then filtered through Whatman no. 41 paper under vacuum. After being filtrated, the sample was evaporated at 45°C using rotary evaporator under high vacuum. The resulting extract were used for all the determinations during this study.

2.3. Anthocyanin content

A pH differential method was used to determine the anthocyanin content. Solution of KCl buffer pH 1 and Natrium Acetate buffer pH 4.5 were prepared. Then, the sample concentration was adjusted to 5000 ppm by diluting 50 mg extract with the buffer solution until 10 ml (DF = 10). After that, sample was analysed by measuring the absorbance at 520 nm and 700 nm. The reason for measuring the absorbance at 700 nm is to correct for haze. The determination of the absorbance of a sample can be calculated by:

Total Anthocyanins (mg/L) = \( \frac{(A \times MW \times DF \times 1000)}{(\varepsilon \times l)} \)

Note:
- \( \varepsilon \) = molar absorptivity of cyanidin-3-glucoside (26,900 L/mol.cm)
- \( l \) = width of cuvette (1 cm)
- MW = molecular mass of cyanidin-3-glucoside (449.2 g/mol)
- DF = dilution factor (10)

2.4. Antioxidant activity

In determination of antioxidant activity, the DPPH method is used by measuring the sample at wavelength of 517 nm. The principle of this method is the reaction of the hydrogen which donates hydrogens atom to neutralize free radicals of DPPH and it will turn the color from purple to yellow [5]. These reaction produce non-radical compounds DPPH-hydrazine which is presented in Figure 2.
2.5. Determination of red dye’s stability on temperature and ultraviolet rays
In this study, the extract was diluted with distilled water to 5000 ppm. Then, the treatment of temperature was given at 55˚C and 80˚C for three hours. While in treatment of ultraviolet rays, the samples were placed in a dark room and a place with ultraviolet rays for a day. After treatments, absorbance of samples were measured to evaluate the changes of the intensity of the color.

3. Results and Discussion
3.1. Anthocyanin content
The total pigment in roselle extract was measured by the pH differential method of Giusti and Wrolstad, which used buffer solution of pH 1 and pH 4.5. In strongly acidic solution (below pH 2), the most stable structure for anthocyanin is in the form of red flavlyium cation. While in weakly acid (pH 3-5), the red flavlyium cation mostly converts to the more stable colorless hemiketal or carbinol (Figure 3) [7].

Based on the results of this study, the highest total anthocyanin was found at pH 2 and temperature of 5˚C (80.4 mg/L). In Figure 4, the effect of pH and temperature on anthocyanin contents is presented.
Figure 4 shows that the lower the pH and temperature, the higher the quantity of anthocyanins obtained. At high temperature, it leads to higher anthocyanins degradation because anthocyanins degrade faster at high temperature compared to low temperature [9]. The damages of the heating can occur through two stages. First, hydrolysis happens in anthocyanin glycosidic bonds so the aglycone become unstable, and then the rings of aglycone are opened and become carbinol groups and chalcone [2]. Analysis of variance (ANOVA) was done for every variation to determine the effect of pH and temperature on total anthocyanin. Result of experiments was obtained by using design expert software. It show that the p-value for pH and temperature to total anthocyanins is <0.0001 smaller than 0.05 which means the effect of pH and temperature is significant as an influence variable of total anthocyanins. It also showed that there is an interaction between pH and temperature.

3.2. Antioxidant Activity
Determination of antioxidant activity was analysed by the DPPH method. The principle of this method is to donate hydrogen atoms to neutralize purple radicals of DPPH, so when the absorbance is measured, the colour of DPPH will be reduced and turn to pale yellow or colorless. The higher antioxidant activity indicates that the ability of samples to reduce free radicals is also high [10]. From the results of this study, the highest antioxidant activity is found at pH 2 and 55°C (90.4%), while the lowest antioxidant activity is found at pH 12 and 5°C (80.1%). In Figure 5, the effect of pH and temperature on antioxidant activity is presented.

Figure 5 shows that a temperature of 55°C produced relatively high antioxidant activity because at high temperatures, it would increase the solubility of solute so many compounds like flavonoids and phenols, which were potentially antioxidant sources, could be extracted. Antioxidant activity depends on total electrons that can be donated. The more antioxidants that can be extracted, it will make the more electrons that can be donated so the antioxidant activity become higher. Lower temperature (5°C) decreases the solubility of active compound, thus decreasing the active compound extracted. Antioxidant activity obtained in acidic conditions (pH 2) was relatively higher than other pH conditions (pH 7 and pH 12). According to Chumsri et al, antioxidant activity of roselle extract is also pH dependent, the activity decreases as pH increases [2]. It happens because in acidic conditions, the increasing number of vacoules cell walls broke out so the anthocyanins and other active compounds like flavonoid and phenols could be well extracted. Result of experiments was obtained by using design expert software. It show that the p-value for pH and temperature to antioxidant activity are <0.0001 and 0.0003, it is smaller than 0.05 which means the effect of pH and temperature is significant as an influence variable of antioxidant activity. It also showed that there is no significant interaction between pH and temperatures, because the p-value for AB is 0.5055, greater than 0.05.
3.3. Yield Content
The yield of roselle’s extract was calculated as \( X = \frac{\text{weight of extract after evaporating}}{\text{weight of dried roselle before extracting}} \times 100\% \). Based on the results of this study, the highest yield contents were obtained at pH 2 and a temperature of 55°C (71.6%). In Figure 6, the effect of pH and temperature on yield content is presented.

Figure 6. The Effect of pH and Temperatures on Yield Content

Figure 6 shows that the higher the temperature, the higher yield content obtained. It is because high temperature increases the solubility of the solute, so that more solute was extracted. But in terms of quality, not only anthocyanin compounds were extracted, but also other compounds like a mixture of citric acid and malic acid and phenols. It caused the obtained yield contents to outgrow the yield content at temperatures of 5˚C and 30˚C.

Result of experiments was obtained by using design expert software. It show that the p-value for pH and temperature to antioxidant activity are 0.0073 and 0.0001 respectively, it is smaller than 0.05 which means the effect of pH and temperature is significant as an influence variable of antioxidant activity. It also showed that there is no significant interaction between pH and temperatures, because the p-value for AB is 0.3044, greater than 0.05.

3.4. Determination of Red Dye’s Stability on Temperature
The heat treatment that has been given to this study was at temperatures of 30˚C, 55˚C and 80˚C. The initial transmittance (%T) of roselle extract was 67.5%. The heat treatment on temperatures of 55˚C and 80˚C made the %T of the sample increase to 70.8% and 78.5%, but %T of roselle extract at a temperature of 30˚C did not change which showed anthocyanins are still stable at this temperature. The increasing of %T on roselle extracts at temperatures of 55˚C and 80˚C happened because anthocyanin compounds were degraded thermally. The degradation mechanism of anthocyanin compounds is presented in Figure 7.

Figure 7. The Degradation Mechanism of Anthocyanin Compounds [10]
Figure 8 shows that the increasing of temperature cause the anthocyanin compounds became unstable. In the initial phase, hydrolysis of the glycosidic bond of anthocyanins will occur to form unstable rings of aglycone. Then, at a later stage, the rings of aglycone will be opened and form carbinol and chalcone groups [12]. From the study, heat treatment at temperature of 55˚C did not change the color significantly so it can be concluded that red color from roselle extract is stable at temperature of 55˚C. However, at temperature of 80˚C, it causes discoloration from red becoming pale red. It shows that thermal degradation on anthocyanin occurre at a temperature of 80˚C.

![Figure 8. Determination of Red Dye's Stability on Temperature](image)

3.5. Determination of Red Dye’s Stability on Ultraviolet Rays
This analysis was observed on discoloration of roselle extract that was stored in a dark space and space equipped with ultraviolet rays. From the study, the red color from roselle extract did not change color significantly in dark space treatment, while in the space equipped with ultraviolet rays treatment experienced a significant change in color from red to reddish yellow. It shows that thermal degradation occurs due to the energy of ultraviolet rays that damages the structure of anthocyanins, which the red cation flavium became colorless carbinol [13].

4. Conclusion
The highest total anthocyanin content in the roselle extract is 80.4 mg/L and it was obtained at 5˚C and pH 2. Meanwhile, the highest antioxidant activity and yield content in the roselle extract were 90.4% and 71.6% respectively at 55˚C and pH 2. From this study, it is known that temperature and pH significantly affect total anthocyanin, antioxidant activity, and yield content. In addition, temperature and ultraviolet rays also affect the stability of red color in roselle petals.

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