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Cite as: AIP Conference Proceedings **1911**, 020014 (2017); https://doi.org/10.1063/1.5016007
Published Online: 05 December 2017

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The Study of Temperature and UV Light Effect in Anthocyanin Extract from Dragon Fruit (*Hylocereus costaricensis*) Rind Using UV-Visible Spectrophotometer

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Abstract. This study aimed to determine the total of anthocyanin content in ethanol extract from super red dragon fruit rind. The extraction was affected by temperature and light conditions. The determination of anthocyanin's total content was performed with a variation of pH and analyzed by UV-Visible spectrophotometer. The results showed that the average contained total anthocyanins obtained at room temperature, 40 and 60 ºC were 4.6757, 5.6108, 21.9757 mg/L, respectively. In higher temperatures, it was observed the more anthocyanin extracted. The concentration of anthocyanin extract without UV light was 2.5716 mg/L, it was less than UV light assisted extract, i.e. 5.3770 mg / L.

INTRODUCTION

Dragon fruit is one of the plants that come from tropical climates. Types of dragon fruit that has been cultivated there are four, i.e. dragon fruit white meat (*Hylocereus undatus*), red flesh dragon fruit (*Hylocereus poyrhizus*), dragon fruit is super red (*Hylocereus costaricensis*), and dragon fruit yellow rind and white flesh (*Selenicereus megalanthus*). Dragon fruit growth is influenced by temperature, humidity, soil conditions and rainfall. Red dragon fruit has a higher antiradical ability than white dragon fruit [1]. Dragon fruit in Indonesia is cultivated in Jember, Malang, Pasuruan, Yogyakarta and other areas [2].

About 30-35% of the dragon fruit that is rarely used is the rind, it was only disposed of as waste and caused environmental pollution. This is unfortunate because the dragon fruit rind has several advantages. The rind of dragon fruit contains vitamin C, E, A and active compounds such as alkaloids, terpenoids, flavonoids, thiamine, niacin, pyridoxine, cobalamin, phenolic, carotene and phytoalbumin [3]. In addition, according to Wu et al. [4], the superiority of dragon fruit rind is rich in polyphenols as a source of antioxidants. Dragon fruit rind has an active compound of betacyanin which can bind to free radicals as an antioxidant [5]. Betacyanin in the rind of dragon fruit is a phenolic compound [6]. According to Saati [7], dragon fruit rind that could be extracted with water as the solvent contains 1.1 mg / 100 ml anthocyanin. Anthocyanins can decrease cholesterol level in the blood [8]. In addition, the antioxidant activity on the dragon fruit rind is greater than the flesh, so it could be developed as the potential source of natural antioxidants. It is in accordance with the research conducted by Nurliyana et al. [9] which found that 1 mg/mL of red dragon fruit rind could inhibit 83.48 ± 1.02% of free radicals, while the dragon fruit flesh could inhibit free radicals of 27.45 ± 5.03%.

Based on the description above, it is necessary to be studied about the rind of super red dragon fruit because of high anthocyanin content that has antioxidant activity. The stability of anthocyanin is influenced by several factors such as temperature and light. Anthocyanin pigments (red, purple and blue) become unstable molecules when the temperature and light were changed. This research was performed to develop a product of dragon fruit rind as

Development of Chemical Education in 21st Century Learning
AIP Conf. Proc. 1911, 020014-1–020014-5; https://doi.org/10.1063/1.5016007
Published by AIP Publishing. 978-0-7354-1603-1/$30.00

antioxidant agent and determine the effect of pH to anthocyanin's concentration in ethanol extract using the UV-visible spectrophotometer [10].

EXPERIMENTAL METHODS

The material used in this research was super red dragon fruit obtained from Local Market in Sleman, Yogyakarta. The chemicals used were 96% Ethanol (Merck), 1% HCl (Merck), Aquadest, NaOH (Merck), KCl (Merck), and Sodium Citrate (Merck). The tools used include a set of glassware, Oven (UN 55), Hotplate (Thermo Scientific Model No. SP131320-30), pH meter (POMETER PH-009 (I)), distillation set, Spectrophotometry UV Vis Single Beam (Thermo Scientific Genesys 20). Analysis of water content on super red dragon fruit rind (Hylocereus costaricensis) using oven method at 105 °C. The empty dish was dried in an oven at 105°C for 1 hour and cooled in a desiccator for 30 minutes. The dry dish is then weighed by the analytical balance and recorded as (W_0). A total of 1,3421-gram samples were inserted into a dry dish then weighed and recorded as (W1). The dish containing the sample was dried in an oven at 105°C for 3 hours. Then put in the desiccator for 30 minutes, then weighed again. The dish is re-introduced into the oven until a constant weight is obtained and is recorded as (W2). The water content in the sample is calculated by the following formula:

$$Water\ content = \frac{w1-w2}{w1-w0}\ x\ 100\% \quad (1)$$

Where:
W_0 = constant dry weight of the dish (g)
W_1 = dish weight and initial sample (g)
W_2 = constant dry weight and dry sample (g)

The process of red dragon fruit rind's extraction with maceration method was done by weighing the rind of dragon fruit that has been fine of 1000 g plus 96% ethanol solvent and 1% HCl with the ratio of 9:1 volume as much as 1000 mL. The distillation process was used to remove solvents. The distillation time was stopped when the solvent is no longer flowing. The resulting extract was weighed. The total anthocyanin content by the pH difference method was determined by determining the dilution factor first. The sample was dissolved with KCl buffer pH 1 until the absorbance was less than 1.2 at a 510 nm wavelength. The 510 nm wavelength was the maximum wavelength for cyanidin-3-glucoside while the wavelength was 700 nm to correct the deposits still present in the sample. If the sample was completely clear then the absorbance at 700 nm was zero [11]. Two solutions with the sample were prepared, in the first sample KCl buffer was used with pH 1 and for the second sample was used Na-citrate buffer with pH 4.5. Each sample was dissolved in a buffer solution based on predetermined DF (dilution factor). Samples dissolved using buffer pH 1 were left for 15 min before measuring, while for samples dissolved with 4.5 pH buffer readily measured after being allowed to mix for 5 min. The absorbance of any solution at wavelength 510 and 700 nm. Measurements were carried out for temperature variations of 40 °C, 60 °C, UV-free conditions and UV exposure conditions. The absorbance of the diluted sample (A) is determined by equation 2.

$$A = (A_{510}-A_{700})_{pH\ 1,0} - (A_{510}-A_{700})_{pH\ 4,5} \quad (2)$$

The content of anthocyanin pigment in the sample is calculated by equation 3.

$$\text{Total Anthocyanin (mg / L)} = \frac{AxBMxDFx1000}{\varepsilon xl} \quad (3)$$

Where :
BM = molecular weight of Cyanidin-3-glucoside = 449.2 g / mol
DF = dilution factor
Ɛ = molar absorptivity cyanidin-3-glucoside = 26.900 L / (mol.cm)
l = cuvette thickness (1 cm)

RESULT AND DISCUSSION

The result of water content analysis on super red dragon fruit rind obtained a constant weight of sample equal to 0.0524 g to obtain a moisture content of 96.10% This is in accordance with Kristanto [2] who found that the water content of the dragon fruit rind is about 90.20%. The dragon fruit rind extraction was performed by distillation method using 96% ethanol solvent acidified with 1% HCl. Ethanol is a polar and non-polar solvent because ethanol has a nonpolar ethyl group and a polar hydroxy. The addition of 1% HCl solvent aims to provide an acidic atmosphere in the maceration process because anthocyanin compounds are more stable in acidic conditions. The result of maceration of 1000 g of super smooth red dragon fruit rind with 1 L solvent (96% ethanol acidified with 1% HCl) with a volume ratio of 9:1 yielded 7.8046 g ethanol extract that is dark brown.

The determination of total anthocyanin content of red dragon ethanol extract was done by pH difference method according to Giusti and Wrolstad [10]. The predetermined dilution factor was 70 dilutions with an absorbance of 0.447 at 510 nm wavelength. Based on the calculation result, the average total anthocyanin content at room temperature, 40, and 60°C, under conditions without UV light and UV light exposure conditions respectively 4.6757 mg / L; 5.6108 mg / L; 21.9757 mg / L, 2.5716 mg / L and 5.3770 mg / L. Total levels of anthocyanin extract ethanol super red dragon fruit rind presented in Table 1 and 2.

TABLE 1. Temperature Effect on Total Anthocyanin Level

Room temperature					**Temperature 40 °C**					**Temperature 60 °C**				
Absorbance				Total levels of anthocyanin (mg/L)	**Absorbance**				Total levels of anthocyanin (mg/L)	**Absorbance**				Total levels of anthocyanin (mg/L)
Buffer pH 1.0		**Buffer pH 4.5**			**Buffer pH 1.0**		**Buffer pH 4.5**			**Buffer pH 1.0**		**Buffer pH 4.5**		
λ 510 nm	λ 700 nm	λ 510 nm	λ 700 nm		λ 510 nm	λ 700 nm	λ 510 nm	λ 700 nm		λ 510 nm	λ 700 nm	λ 510 nm	λ 700 nm	
0.442	0.125	0.453	0.137	1.169	0.415	0.09	0.442	0.123	7.014	0.417	0.082	0.407	0.094	25.716
0.442	0.125	0.451	0.135	1.169	0.413	0.088	0.447	0.124	2.338	0.414	0.082	0.408	0.093	19.872
0.443	0.126	0.445	0.134	7.014	0.412	0.086	0.447	0.125	4.676	0.417	0.081	0.409	0.093	23.378
0.442	0.126	0.444	0.134	7.014	0.412	0.087	0.445	0.123	3.507	0.415	0.082	0.409	0.094	21.041
0.442	0.125	0.444	0.133	7.014	0.415	0.088	0.442	0.122	8.183	0.415	0.083	0.408	0.093	19.872
Average				**4.676**					**5.143**					**21.976**

TABLE 2. UV Light Effect on Total Anthocyanin Level

Conditions with UV light					**Conditions without UV light**				
Absorbance				**Total levels of anthocyanin (mg/L)**	**Absorbance**				**Total levels of anthocyanin (mg/L)**
Buffer pH 1,0		**Buffer pH 4,5**			**Buffer pH 1,0**		**Buffer pH 4,5**		
λ 510 (nm)	**λ 700 (nm)**	**λ 510 (nm)**	**λ 700 (nm)**		**λ 510 (nm)**	**λ 700 (nm)**	**λ 510 (nm)**	**λ 700 (nm)**	
0.442	0.117	0.452	0.129	2.3378	0.445	0.122	0.588	0.261	-4.6757
0.444	0.116	0.447	0.128	10.5203	0.449	0.12	0.589	0.265	5.8446
0.443	0.115	0.452	0.128	4.6757	0.448	0.121	0.589	0.266	4.6757
0.442	0.117	0.449	0.128	4.6757	0.446	0.122	0.587	0.266	3.5068
0.442	0.116	0.449	0.127	4.6757	0.447	0.12	0.588	0.264	3.5068
Average				**5.3770**					**2.5716**

Table 1 and 2 showed that in red leaf dragon fruit extracts have different total levels, this is due to several factors such as in the sample preparation process, the influence of temperature and UV light, and the habitat of the red dragon fruit sample used. In the sample distillation process using a high enough temperature that is 60-70°C so that the process affects the stability of anthocyanin because it is done continuously until the extract obtained. In accordance with Niendyah [12], the stability of anthocyanin is influenced by several factors such as temperature, pH, light and oxygen changes, and other factors such as metal ions. In addition, the indirect sample analysis process

was conducted at that time, so that the conditions and storage period of the sample caused the anthocyanin compound to be degraded. The effect of temperature and UV light in dragon fruit extraction could be seen in Fig. 1 and 2 which showed that the extract of red dragon fruit rind using ethanol was affected by temperature and UV rays.

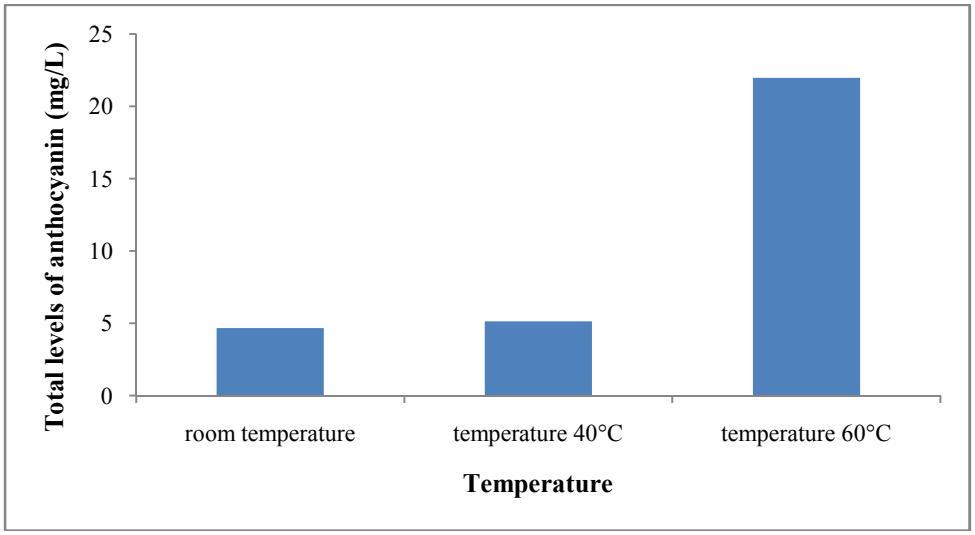

FIGURE 1. Temperature effect on total anthocyanin level

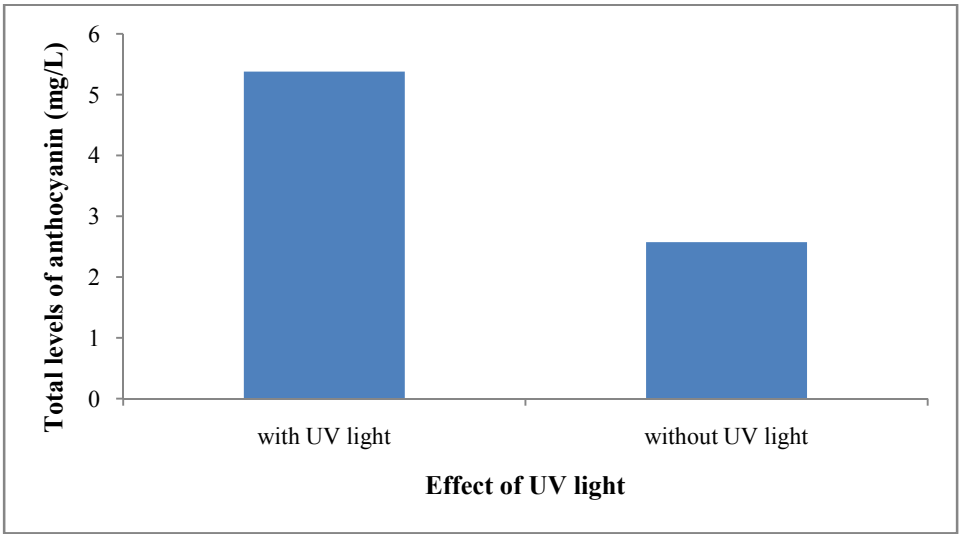

FIGURE 2. The Effect of UV on total anthocyanin level

Based on the result, Fig. 1 showed that the total anthocyanin content is influenced by temperature. The highest anthocyanin level was obtained at 60°C at 21.976 mg/L. However at temperatures greater than 70 °C anthocyanins will experience significant degradation [13]. Based on the diagram between the influence of UV light with the total anthocyanin content in Fig. 2, the anthocyanin content without UV light was 2.5716 mg/L and the anthocyanin level exposed to UV light was 5.3770 mg/L. The results of the measurement of anthocyanin extract of red dragon fruit rind showed that levels increased due to the influence of UV light. Siregar and Nurlela [14] stated that the energy coming from UV light is called insolation. This insulation consists of radiant rays composed of various wavelengths. Shorter wavelength light will produce certain phytochemical effects and are able to accelerate the oxidation process of biomolecules as well as fruit maturation processes. Anthocyanins also have a strong tendency to absorb visible

light and radiant energy of light causing photochemical effects on the visible spectrum and resulting in discoloration [15].

CONCLUSION

From the results of the research, it can be concluded that the total anthocyanin content obtained based on the effect of room temperature is 4.6757 mg/L, 5.6108 mg/L at 40 °C, 21.9757 mg/L at 60 °C, the condition without UV light of 25716 mg/L and the condition with UV light of 5.3770 mg/L. So the temperature variation of 60 °C and the conditions exposed to UV light in the determination of total levels of anthocyanin in extract ethanol red dragon fruit rind gives maximum results.

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