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Stability Study of Betacyanin Extract from Red Dragon Fruit (*Hylocereus polyrhizus*) Peels

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Abstract

Dragon fruit (*Hylocereus polyrhizus*) or buah naga is one of the tropical fruits under the cactus family, *Cactaceae*. Dragon fruit becoming popular in Indonesia due to its attractive color, sweet, juicy pleasant taste and also the plant is beautiful. The peels of red dragon fruit is the potential sources of betacyanins which responsible for the red-violet color. The objective of this study was to evaluate the stability of betacyanin extract from red dragon fruit peels. In this study, the betacyanin extract from the peels of red dragon fruit was extracted by methanol and water. The stability of this pigment was evaluated by monitored the effects of storage time and pH by spectrophotometer at wavelength 538 nm. This study was also investigated the kinetics degradation of betacyanin extract using the accelerated reaction method. Results analysis of red dragon fruit peels extract shown that for five hours in room temperature, betacyanin content in methanol extract was decreased about 10.44%, meanwhile betacyanin content in water extract was decreased about 22.58%. Betacyanin content was obtained from peels which extracted by methanol pH 5 (515.20 µg/100 g) higher than betacyanin content in water pH 5 (491.16 µg/100 g). Arhenius data showed that betacyanin extract in water follow the first-order kinetic model with its half life time (t ½) at 25°C was 23 hours and 90%-shelf life was 76 hours.

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*Keywords:*betacyanin, dragon fruit, stability, Arrhenius

1. Introduction

Dragon fruit (*Hylocereus polyrhizus*) is a member of *Cactaceae* family from the genus *Hylocereus*. The peels of this fruit have the attractive color and when ripened its flesh has red-purple color with black seeds interspersed. The

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deep purple color of the pulp is contributed by a set of pigments known as the betalains which are nitrogen-containing pigments [1]. Dragon fruit or "buah naga" becoming popular in Indonesia due to its attractive color, sweet, juicy pleasant taste and also the plant is beautiful. Some studies have shown that a mature dragon fruit contains considerable amount of total soluble solids, rich in organic acids, protein and other minerals like potassium, magnesium, calcium and vitamin C. Furthermore, the dragon fruit has been reported can exhibit high antiradical activities with the presence of phenolic compounds [2]. Some synthetic food colorants derived from minerals may be hazardous to human health. Natural colorants from plant sources are becoming interest from both food manufacturers and consumers in order to replace synthetic colorants. Natural food colorants have been criticised for being more expensive and less stable. However, as the list of approved artificial colors has diminished under increasing regulation by the FDA [4]. Some major categories of plant pigments are betalain, anthocyanins and other flavonoids, carotenoids and chlorophylls. Betalain is water-soluble and can be classified into two main groups, betacyanin is responsible for the red-violet color and betaxanthin is responsible for the yellow-orange color [3]. The potential sources of betacyanins are red beet, cactus pear, pitaya and Amaranth. The most common sources of betalains is the red beet, but it is restricted by its earthy smell as well as considerable nitrate level [4].

Unlike synthetic coloring agents, most of the natural colorants are easily degraded, heat-labile and low in stability due to their natural structure. Betalains possess anti-oxidant properties which are prone to oxidation. Thus, prevention of oxidation which occurs during extraction and storage is crucial [3]. Betacyanins and anthocyanins are chemically related, the methods of anthocyanin extraction can therefore be applied for betacyanin. Compared to polar anthocyanins, betacyanins are more hydrophilic. In more specific, they can dissolve in three common polar solvents such as water, methanol and ethanol [5]. The objective of this study was to evaluate the stability of beatcyanin extract from red dragon fruit peels. In this study, the betacyanin extract from the peels of red dragon fruit (*Hylocereus polyrhizus*) was extracted by methanol and water. The stability of this pigment was evaluated by monitored the effects of storage time and pH by spectrophotometry method. This study was also investigated the kinetics of degradation of betacyanin extract from peels of red dragon fruit.

Nomenclature

g	weight (gram)
^{o}C	temperature in Celsius
UV-Vis	Ultra Violet-Visible
λ	wavelength
L	path length
E	mean molar absorptivity
MW	molecular weight
R	gas constants
A	frequency factor
K	degradation rate constants
T	absolute temperature (K)
E	activation energy

2. Materials and Methods

2.1. Materials

The dragon fruit *(Hylocereus polyrhizus)* which used in this study was purchased from a supermarket in Bandung Indonesia. The peels were separated from the pulp using a stainless steel knife.

2.2. Preparation of betacyanin extract

Distillated water and methanol extractions were carried out in order to investigate which type of extraction could exhibit the highest amount of betacyanin pigments. Exactly 100 g of peels was weighed and 400 ml of each

solvent (water, methanol, and acidic water or methanol) was added in a beaker. The extraction was carried out by maceration process for 24 hours in a refrigerator. The extract was then filtrated by using a filter paper no 41. Filtrate was stored in a refrigerator for further analysis. Due to the stability of extract, the samples were prepared freshly.

2.3. Determination of total betacyanin content in samples

Absorbance for all extract samples were measured at 538 nm using a spectrophotometer UV-Vis. The absorbance readings obtained was used to calculate the total betacyanin concentration sample. The quantification of betacyanin was described by Lim *et al.* [6]. The betacyanin content (µg/100 g of fresh weight) was calculated using Equation:

$$\frac{A\,(MW)\,x\,V\,x\,(DF)\,x\,1000}{E\,L\,W}\,100 \qquad (1)$$

where A = absorbance at 538 nm (λ max), L (path length) = 1.0 cm, DF = dilution factor, V = volume extract (mL), W = fresh weight of extracting material (g). For betanin, E (mean molar absorptivity) = 6.5 x 10^4 L/mol cm in H_2O and MW (molecular weight) = 550 g/mol.

2.4. Pigment stability analysis

- Effect of storage time: 5 mL of extract was placed in each of reaction tubes at room temperature. The absorbance of extract was monitored each hour for 5 hours.
- *Effect of pH:* The effect of pH on the yield of total betacyanin was studied at pH values; 1.0, 2.0, 3.0, 4.0 and 5.0. pH of samples was adjusted by adding ascorbic acid until the desired of pH.

2.5. Degradation rate analysis of betacyanin extract

Degradation rate of extract was analyzed by modification of Robert *et al.* [7] method. 5 mL of betacyanin extract in distillated water were placed in each of reaction tubes. These tubes were placed in a water bath at 50, 60 and 70 ^{o} C and the absorbance was observed for every 30 minutes until 120 minutes. The absorbance was determined at 538 nm. Degradation rate constants (k) and half time (t½) of betacyanin was calculated using Arrhenius model:

$$\ln k = \ln A - \frac{E}{RT} \qquad (2)$$

R = gas constants (1,987 calorie/K mol)
A = frequency factor
K = degradation rate constants
T = absolute temperature (K)
E = the activation energy
t ½ = 0,693/k
Life time 90% at 25^{o}C = 2.303/k

3. Results and Discussion

The stability of natural pigments generally less stable than synthetic colorants. Eventhough, these pigments mostly have more attractive color and its application in food colorants more reccomended due to its safety. Based on chemical structure, betacyanin more soluble in water than in non-polar solvents and this characteristic helps extraction and separation processes [5]. In this study, betacyanin extract form peels of dragon fruit was evaluated the solubility and its stability for five hours storage time. Betacyanin extract from dragon fruit peels was prepared one day before analysis to avoid the degradation of this pigment. Data analysis shown that the solubility of betacyanin in methanol was higher than in water. The initial betacyanin content in methanol was 531.45 µg/100 g and the initial betacyanin content in water was 394.35 µg/100 g of peels. Moreno *et al.* [8] reported that methanol has generally been used to extract betacyanins, primarily for analysis purpose. Based on monitoring for five hours in room temperature, betacyanin content in methanol was decreased about 10.44%, meanwhile betacyanin content in water was decreased about 22.58%. The stability of betacyanin from red dragon fruit peels which extracted by methanol and water was presented in Figure 1.

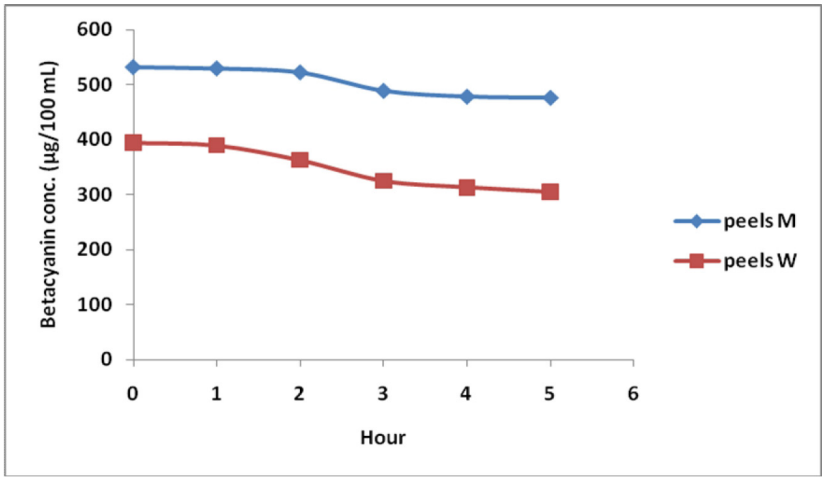

Figure 1. The stability of betacyanin from red dragon fruit peels, kept in room temperature for 5 hours (peels M: peels extract in methanol, peels W: peels extract in distillated water)

The effect of pH on the yield of total betacyanin was studied by extraction the peels with methanol and water at pH 1, 2, 3, 4 and 5. Figure 2 shows that the total betacyanin content obtained from 100 g peel in 400 mL of water or methanol at different pH. Total betacyanin content which extracted by methanol pH 1, 2, 3, 4 and 5 were 188.54, 222.73, 279.94, 379.46 and 515.20 µg/100 g of peels. Meanwhile, Total betacyanin content which extracted by distilled water pH 1, 2, 3, 4 and 5 were 216.40, 265.38, 313.80, 378.10 and 491.16 µg/100 g of peels. The highest yield of betacyanin content was obtained from peels which extracted by methanol pH 5. Herbach *et al* [9] reported that betalain pigments favor a pH range of pH 4 to 6 in the presence of oxygen and also under anaerobic condition. According to K.V. Harvaidaran *et al* [1] study, showed that pH 5 is the pH condition to extract and obtain highest yield of betalain content was 25.74 mg/L of dragon fruit peels extract in water. Betalains were first extracted from the red beet (*Beta vulgaris*). The betalain extract contains red and yellow pigments, namely betacyanin and betaxanthins, respectively[1].

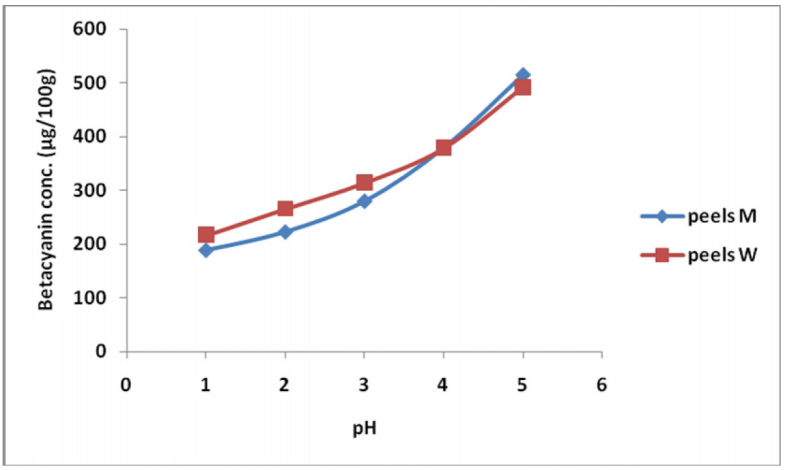

Figure 2. Total betacyanin content from red dragon fruit peels extract in different pH of solvents
(peels M: peels in methanol, peels W: peels in distillated water)

To study the degradation rate of betacynin and its kinetic reaction, the extract was analyzed its stability at 50, 60 and 70°C for two hour. Red dragon fruit peels was extracted by water because of high temperature that used in this experiment. The high temperature was used in this experiment in purpose to accelerate the time of experiment. The accelerated reaction method (Arrhenius) was used to predict half life ($t_{½}$) of this pigment. The most common method to estimate the Arrhenius parameters is the classic successive two-steps ordinary linear least squares fit. In this method the regression of natural log of percentage retention for betacyanin degradation vs. time is done at each temperature, to estimate the degradation rate constants (k). The second step is the regression of ln k vs. 1/T to obtain the estimates of ln k_0 and Ea/R [10]. A plot of natural log of percentage retention for betacyanin degradation vs. time was shown on Figure 3. The data shows the natural logarithm of the absorbances of betacyanin extract vs. time. The degradation of this pigment followed first-order behaviour for all the temperature studied. The correlation coefficient was used as a parameter to determine the reaction order.

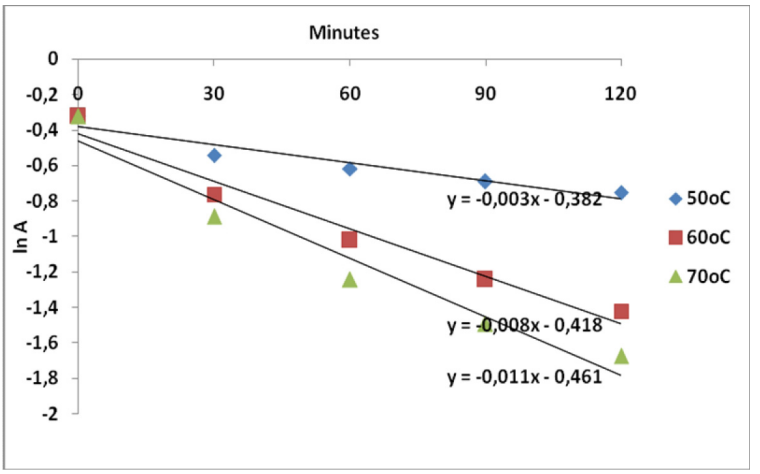

Figure 3. Degradation rate of betacyanin extract of red dragon fruit peels

On Figure 4 shows the correlation of natural logarithm of degradation rate constants (ln k) vs. 1/T (K). On the data shows the equation of Linear Least Squares data was y = -7231 x + 16,68, furthermore this equation was used to

determine the Arrhenius parameter values to predict the half life (t ½) and life time 90% of betacyanin extract at 25°C (Table 1).

Table 1. Arrhenius parameter of betacyanin extract of peels red dragon fruit

Parameter	Value
Activation energy (Ea)	14.37 kcal/mol
Intercept (ln A)	16,68
r^2 (Arrhenius plot)	0,929
Half life (t ½) at 25°C	23 hours
Life time 90% at 25°C	76 hours

The accelerated reaction method (Arrhenius) has been applied to predict the stability of food products, drugs and cosmetics. The data on Table 1 shows that betacyanin extract of red dragon fruit peels was sensitive to thermal process due to the degradation of its compounds. The high temperature can accelerate betanin, the main structure of betacyanin, to be regenerated into two products, betalamic acid and cyclodopa-5-Oglycoside, in unstable form. During heat processing, betanin may be degraded by isomerisation, decarboxylation or cleavage, resulting in a gradual reduction of red colour, and eventually the appearance of a light brown colour [11].

Conclusion

The stability of betacyanin extract from red dragon fruit peels was influenced by the kind of solvent, pH and temperature. Results analysis shown that for five hours in room temperature, betacyanin content in methanol extract was decreased about 10.44%, meanwhile betacyanin content in water extract was decreased about 22.58%. Betacyanin content was obtained from peels which extracted by methanol pH 5 (515.20 µg/100 g) higher than betacyanin content in water pH 5 (491.16 µg/100 g). Arrhenius data showed that betacyanin extract in water follow the first-order kinetic model with its half life time (t ½) at 25°C was 23 hours and 90%-shelf life was 76 hours.

Acknowledgements

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