Effect of glucose, sucrose, and lactose solution on the stability of betacyanin pigment from red dragon fruit (Hylocereus polyrhizus) peels

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Abstract. People use peels, stems, leaves, and fruits of plants as a natural colorant. One part of fruit peel that has potentially used as a food colorant is red dragon fruit peels. The pigments found are betacyanin, but betacyanin compounds are easily degraded by heat, pH, and light. Degradation of betacyanin forms betalamic acid compounds (yellow) and cyclo-DOPA acids (colorless). In this work, an attempt was made to increase the stability of betacyanin pigment against temperature by adding stabilizer such as glucose, sucrose, and lactose. Betacyanin extract was obtained from extraction using water solvent at pH 6 with a ratio of 1:4 (w/v). Extract was diluted and added stabilizer at concentration 0 ppm, 100 ppm, and 150 ppm. The solution was heated in a water bath at temperature of 60 °C for 1 hour. The content of betacyanin was examined by a UV-VIS spectrophotometer at a wavelength of 534.8 nm every 15 minutes. The retention value for 150 ppm of lactose was the greatest of all the stabilizer with the value of 61.95%. Pigment betacyanin was degraded more slowly in a lactose solution. The highest half-life time increased by 24.24% for dissolution in 150 ppm lactose. Thus, lactose has the potential to increase the stability of betacyanin.

1. Introduction

People use the peels, stems, leaves, and fruits of plants to give a certain color to food. Food colorant has role to influence the nature or form of food, so that the appearance change and make consumers interested [1]. Natural food colorant began to be displaced by the discovery of synthetic dyes. Most synthetic dyes will provide a brighter and more interesting color effect on food, however it has been found that some synthetic dyes contain carcinogenic azo dyes (aromatic amines) [2]. Research by Amin in 2010 concluded that tartrazine and carmoisine give bad effects in the liver and kidney even in low doses [3].

Today, the public as consumers are smarter, more careful, and more observant in choosing food colorant products. The public believes that products from natural ingredients are healthier and safer for consumption in the long term of life. Thus, this research will be conducted to improve the stability of natural pigment especially betacyanin from red dragon fruit peels.
Dragon fruit belongs to the cactus plant group of the Cacteceae family with the genus *Hylocereus*. This cactus plant is native to South America from the tropical forests of Mexico. Some of the classifications are white dragon fruit (*Hylocereus undatus*), red dragon fruit (*Hylocereus polyrhizus*), and yellow dragon fruit (*Selenicereus megalanthus*) [4]. Dragon fruit itself has a characteristic shape of an elliptical fruit with an average weight of around 400-500 grams with a skin weight ranging from 20-30% of the whole fruit. The texture of the dragon fruit peel is thick and smooth, which is about 3 mm - 4 mm [5]. The image of dragon fruit peel can be seen in figure 1.a. Red dragon fruit is usually used for natural red colorant because of its betacyanin content. Besides that, dragon fruit is usually packaged in the form of juice and fruit chips, while the dragon fruit peel is thrown away. The skin contains 6.7mg of betacyanin equivalent/100 g dry weight [6]. Betacyanin is classified as betalains compounds which have red pigments. Betacyanin contains nitrogen glycosylated compounds responsible for the red color and is chemically distinct from the anthocyanins, which include many red and pink plant pigments. The chemical structures of betacyanin can be seen in figure 1.b.

![Image of Dragon Fruit Peels Used in This Study](a)

![Chemical Structure of Betacyanin](b)

**Figure 1.** (a) Image of Dragon Fruit Peels Used in This Study (b) Chemical Structure of Betacyanin

Betacyanin extraction can be done using polar solvents, such as ethanol, methanol, and water [6]. However, Betacyanin can be easily degraded because of heat, pH, and light. Some of the derivatives compounds are betalamic acid compounds (bright yellow), betanidin (bright yellow), and cyclo-DOPA-5-O-β-glucoside acid (colorless) [7]. Color degradation occurs because of bond cleavage in Nitrogen atom, dehydrogenation in the carboxyl group, and deglycosylation[8]. One way to improve the stability of natural colorant is by using a stabilizer [9]. For example, by adding 700 ppm of ZnCl₂ chlorophyll pigment was more stable during heating at 85 °C[10]. Stabilizer Zn²⁺ ion can substitute the Mg²⁺ bonding in porphyrin rings [11]. For betacyanin, recent studies show that adding ascorbic acid by 0.25% (w/v) can increase the stability during storage at 5 °C for 28 days with 79.2% pigment retention [7]. Ascorbic acid can form cation radicals that can protect betacyanin from degradation [12].

Glucose, sucrose, and lactose are classified as sugar. Glucose is classified as a monosaccharide while sucrose and lactose are classified as disaccharide. Glucose and lactose can be classified into reducing sugars because both of them have free anomic carbon which can be used by other molecules to get reduced (red circle) as shown in figure 2 [13]. All three types of sugar are easily soluble in water because they have lots of polar hydroxyl groups which can make hydrogen-bond with water molecules. The more bonding, the lower the water activity. Water activity can be defined as a measure of how efficiently the water present can take part in a chemical (or physical) reaction [13]. Therefore, this present research was conducted to maintain the stability of betacyanin pigment from dragon fruit peel at higher temperatures by adding glucose, sucrose, and lactose.
2. Materials and Method

2.1. Materials

The materials used in this study was the dragon fruit peels. The dragon fruit was purchased from Market around Yogyakarta City, Indonesia. The flesh and peels were separated using a knife. Glucose, sucrose, and lactose powder Proanalysis (PA) was produced by Merck. For other reagents and materials were purchased from CV Sigma Aldrich Yogyakarta.

2.2. Preparation of Dragon Fruit Peel

Dragon fruit peel was washed with water at room temperature to remove dirt. The peel was cut into small pieces using a knife. Then the peel was blended to a smaller size.

2.3. Betacyanin Extraction from Dragon Fruit Peel

Method for betacyanin extraction refers to Priatni et al. and Tang et al. with a slight modification. Dragon fruit peel was extracted using distilled water which is adjusted to pH 6. The ratio used in this experiment was 1:4 (w/v). Extraction was carried out at room temperature for 3 hours on a shaker. After extraction, the sample is filtered using filter paper and centrifuged at a speed of 3000 rpm for 15 minutes. The extract is stored in the refrigerator to maintain the stability of the betacyanin extract. All samples used are fresh extract.

2.4. Stability of Betacyanin Pigment

In this study, to determine the effect of glucose, sucrose, and lactose against the stability of betacyanin, 10 mL of extract was diluted into 90 mL buffer solution pH 6. Three dilutions extract were made and added by glucose to a concentration of 0 ppm, 100 ppm, and 150 ppm. With the same ways extract solution were made and added by sucrose and lactose. All the solution was heated in a water bath at 60 °C for 1 hour. The betacyanin content was analyzed every 15 minutes. As a comparative study, a blank solution (0 ppm), 100 ppm and 150 ppm of ascorbic acid used as a comparative solution. This is due to the research Determination of Betacyanin Content by Norziahin 2007 that ascorbic acid can be used as a stabilizer. Therefore, ascorbic acid is used to test the effectiveness of glucose, sucrose, and lactose as stabilizers. Betacyanin content was analyzed using spectrophotometer UV-Visible. The total of betacyanin was carried out applying the equation [14]

\[
BC = \frac{Ax DF x MW x V x 1000}{\epsilon x L x W} \times 100
\]

Where, BC is the betacyanin contents in µg/100 gram fresh weight, A is the absorbance value at wavelength 538.6 nm, DF is the dilution factor, MW is the molecular weight (550 g/mol), V is mL volume extract, \(\epsilon\) is the molar extinction coefficient of (65,000 L/mol/cm), and L is the path length of the cuvette (1.0 cm).

3. Result and Discussion

The major goal of the presented study was an estimation of betacyanin stability in different concentrations of glucose, sucrose, and lactose solution upon thermal treatment at 60 °C. Additionally, the concentration used for examined was 0 ppm, 100 ppm, and 150 ppm. Absorption spectra of Hylocereus polyrhizus peels of betacyanin solutions were recorded using a UV-visible

![Figure 2](image-url)
spectrophotometer. Logger Lite 1.6.1 was used to evaluate the absorbance of betacyanin pigment at varying wavelengths (λ_{max}). The solvent used for extraction was distilled water because betacyanin was soluble at polar solvents rather than non-polar solvents [15]. In addition, the selection of solvents was also based on the ease of solvency to be obtained and food safety criteria to be used as food colorant. The absorbance profile analysis results with a spectrophotometer were presented in figure 3.

![Figure 3](image-url)

**Figure 3.** The Absorbance Profile of Betacyanin Pigments at Various Wavelengths (a) Stabilizer Concentration of 100 ppm (b) Stabilizer Concentration of 150 ppm

The peak of the betacyanin pigment lies at a wavelength of 538.6 nm. Absorbance in a blank solution for data variation of 100 ppm was 0.124 and for data variation 150 ppm was 0.193, thus the initial betacyanin concentration was 146.3 µg / 100 g peels and 151.8 µg / 100 g peels. After heating at 60 °C, a change in the peak profile occurs. Absorbance at wavelength 538.6 nm decreased while absorbance at wavelength 394.6 nm and 422.3 nm increased. The absorbance at 422.3 nm was spectrum for betalamic acid (yellow color) [16]. During the heating process, breaking bonding occurred and caused a color reduction to pale red or turns to bright yellow. This indicates that betalamic acid and cyclo-DOPA-5-O-β-glycoside (colorless) compounds were formed. Heating might cause the electronic kinetic energy to increase so that the electron will be excited and cause the breaking of bonding. Termination of bond occurs in the central nitrogen atom [17]. Moreover, heating in acidic solution could cause dehydrogenation of carboxyl to form neobetanin (yellow color) and deglycosylation of glucose to form betanidin (yellow). The degradation mechanism of betacyanin is shown in figure 4.

In this study, the degradation was evaluated at pH 6 because betacyanin was more stable in acidic conditions (pH 5-6) [18]. At lower pH value, betacyanin pigments had light pinkish. Acid condition induced re-condensation of betalamic acid with the amine group of the addition residue [19]. On the other hand, at higher pH value had a light yellow color. This condition causes aldime bond hydrolysis so that betacyanin change to betalamic acid (light yellow) [20]. The kinetic degradation of betacyanin followed first-order reaction, as previously demonstrated by Sri Priatni et al [21]. **Figure 5** showed the plot of the natural logarithm of concentration versus time.
The concentration profile (ln Ca) over time shows that the rate of degradation at this temperature follows first-order reaction. Data trends for each figure were linear. From these data, the gradient of each line indicates the value of the constant rate of degradation (k). The value of k at a concentration of 100 ppm stabilizer was 0.0173 minute\(^{-1}\) for blank, 0.0171 minute\(^{-1}\) for ascorbic acid, 0.0163 minute\(^{-1}\) for glucose, 0.0147 minute\(^{-1}\) for sucrose, and 0.0142 minute\(^{-1}\) for lactose. This value was greater than the constant rate for the 150 ppm stabilizer. Sequentially, the value of the degradation rate constant for the 150 ppm stabilizer were 0.0082 minute\(^{-1}\), 0.0069 minute\(^{-1}\), 0.0073 minute\(^{-1}\), 0.0074 minute\(^{-1}\), and 0.0066 minute\(^{-1}\). The smaller the value of k, the slower the rate of degradation. The smallest value was 150 ppm of lactose as a stabilizer. This might indicate that the addition of lactose at a concentration of 150 ppm could inhibit the rate of betacyanin degradation to its derivative compounds. At a concentration of 150 ppm, the half-life time value increased by 18.84% for ascorbic acid, 12.33% for glucose, 10.81% for sucrose, and 24.24% for lactose.
Based on Figure 6, it could be concluded that the addition of ascorbic acid, glucose, sucrose, and lactose give a positive effect on the stability of betacyanin pigment against temperature. Stabilization increases with the addition of concentration. In acidic solution, sucrose and lactose can be hydrolyzed into two compounds. Hydrolysis of sucrose yields glucose and fructose, while lactose is hydrolyzed to glucose and galactose [22]. Those data from Figure 6 show that monosaccharide gives lower stability rather than disaccharide. A high concentration of disaccharide solution presents more combinations of sugar molecules and water, hence water activity reduced [23]. Lactose gives higher stability than sucrose because of its reducing characteristic. The retention value for 150 ppm of lactose was the greatest of all the stabilizer with the value of 61.95%. Therefore, lactose can avoid the degradation of betacyanin.

4. Conclusions
The degradation of the betacyanin causes the red color to turn yellow. Adding glucose, sucrose, and lactose solution give a positive effect on the stability of betacyanin pigment. The highest stability was obtained by the addition of 150 ppm lactose so that the half life time value at 60 °C heating increased by 24.24%.

5. Acknowledgments
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