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Thermal and photostability of betacyanin from dragon fruit (*Hylocereus polyrhizus*)

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ABSTRACT

Betacyanin derived from red beetroot is a permitted natural colourant, which has been widely used in food systems. However, a high dosage of betacyanin will give an undesirable odour and unfavourable earthy flavour due to the presence of geosmin. An alternative source to replace betacyanin in beetroot is to extract betacyanin from red purple pitaya (*Hylocereus polyrhizus*). However, this natural pigment is easily degraded under high temperature and sunlight. This research aims to determine the photostability and heat stability of the betacyanin using Response Surface Methdology at different pH, temperature, light exposure, and ascorbic acid concentrations. The parameters involved were temperature, light, pH, and ascorbic acid at different ranges 65-95°C, 10-70 h, pH 3-7 and 0.1 g/mL-1.5 g/mL, respectively. The optimal betacyanin thermal stability was obtained at 77.428°C, pH 3 and 1.000 g/mL of ascorbic acid while the optimal photostability was obtained at 44.726 h, pH 5.391 and 0.629 g/mL of ascorbic acid. Betacyanin also have high total phenolic content of 13.607 – 18.071mg GAE/mL and antioxidant activity based on DPPH scavenging activities of 546.549 µg/mL and 519.05 µg/mL. To conclude, this study proposed the optimum condition to retain the highest amount of betacyanin were temperature lower than 77.428°C, pH lower than pH 5.5 with 0.5 – 1.000 g/mL of ascorbic acid. This information offers a useful guideline for food manufacturers to create the best storage condition for their products containing betacyanin.

Keywords: Betacyanin, Response surface methodology, Thermal stability, Photostability

INTRODUCTION

Dragon fruit or known as red pitaya belongs to the Cactaceae family from the subfamily Cactoidea of the tribe Cactea. *Hylocereus polyrhizus* (*H. polyrhizus*) is one of the dragon fruit species that is a member of the Cactaceae family and one of the 13 families in the plant kingdom. The red pigment of *Hylocereus polyrhizus* (*H. polyrhizus*) is contributed by betacyanin, a compound from a set of water-soluble nitrogen-containing pigments known as betacyanin (Rebecca et al., 2010). Betacyanin act as a natural dye in food product while providing antioxidant

properties (Naderi et al., 2010). Antioxidant helps prevent oxidative damage of reactive oxygen species to the biological macromolecules by donating a hydrogen atom to a free radical molecule (Lobo et al., 2010). Betacyanin is a red pigment previously found in beetroot. However, beetroot has an earthy odour and the extracted dye from this source contains an unpleasant odour (Esquivel et al., 2007). Betacyanin is also presents in dragon fruit, which is believed to be more suitable than beetroot for food application. Meanwhile, synthetic colour such as Allura Red has been reported with various health problems (Renita et al., (2023), which requires a safer alternative like natural colourant.

This natural betacyanin pigment covers red purple colour and has high stability towards pH and temperature. However, there are several factors affecting betacyanin stability such as pH, temperature, light, water activity, oxygen, metals, and antioxidant. Betalain stability is affected by light such as ultraviolet (UV) or visible light, as it excites the betalain chromophores that are π electron to a higher energy state (π*), resulting in lower activation energy and higher molecule reactivity. According to Torres et al., (2020), the highest pigment retention was observed at 4°C while the lowest was observed at 70°C. The sample of dragon fruit (*H. polyrhizus*) have better betacyanin content when in dark condition compared to when exposed to light conditions (Wong and Siow, 2015). Betacyanin prefers acidic pH and is stable between pH 3 to 7 whereas it would be readily degraded beyond this range (Woo et al., 2011). Red-fleshed dragon fruit have an initial pH of around pH 5. pH 4 to 6 were reported as the optimal pH for betacyanin stability (Kunnika and Pranee, 2011; Vaillant et al. 2005). Herbach et al. (2006) reported that the best results of betacyanin stability were obtained when red-fleshed dragon fruit juice was heated at pH 4 rather than pH 6. The betacyanin pigment stability will be improved when the supplementation of ascorbic acid concentration at 0.25% and the addition of ascorbic acid was evaluated at different temperatures (5 and 25 °C) over a storage period of 28 days that causes the reduction of pigment retention over times when there is excess supplementation of ascorbic acid to this pigment (Woo et al., 2011). Based on Wong and Siow (2015), the addition of 0.25% of ascorbic acid gives the highest betacyanin content to the extract of dragon fruit.

The pigment stability becomes an important consideration for optimising utility for both food colourant and antioxidant activity. Therefore, this research aims to determine the photostability and heat stability of the betacyanin using RSM at different pH, temperature, light exposure, and ascorbic acid concentrations.

MATERIALS AND METHODS

Preparation of sample

Dragon fruit (*H. polyrhizus*) bought from supermarket was rinsed with water and let to air dried. The peel was then separated from the flesh and cut into small pieces for extraction purposes.

Extraction of Betacyanin

A total of 10 g of dragon fruit peel was weighed and transferred into a mortar and pestle. The sample was ground in 100 mL of methanol and the paste was transferred into a 50 mL centrifuge tube. Another equivalent volume of methanol was added to wash the residue. The mixture was stirred with a vortex for 1 min and then centrifuged at 18,000 rpm for 20 min. Supernatant was poured into an amber bottle and kept in a dark vessel (Kunnika and Pranee, 2011).

Determination of Total Betacyanin Content in the dragon fruit flesh

Absorbance for betacyanin was measured at 538 nm using spectrophotometer UV-Vis (Agilent Technologies, Santa Clara, CA). The absorbance readings obtained were used to calculate the total betacyanin concentration sample. The quantification of betacyanin was described by Woo et al. (2011).

Response Surface Methodology (RSM)

The effects of 4 independent variables (temperature, light, pH, and ascorbic acid) on the response variable (total betacyanin content) were determined using Central Composite Design (RSM). Rotatable designs provide

constant prediction variance at all points that were equidistant from the design centre. A total of 18 runs for both thermal and photostability data were performed. Table 1 shows the independent variable with the range of its parameter for the thermal and photostability data that need to be inserted in the RSM.

Table 1. The independent variables with a range of parameters for thermal stability (temperature) and photostability (light exposure)

Factors	Units	Range	
		Low	High
Temperature (Thermal stability)	°C	65	95
Light exposure (Photostability)	h	10	70
pH	-	3	7
Ascorbic acid	g/mL	0.1	1.5

Determination of Total Phenolic Content

The total phenolic content (TPC) was estimated using the Folin-Ciocalteau method according to Nerdy and Manurung (2018). Both the optimised thermal and photo betacyanin extract (0.5 mL) were added with 7.5 mL distilled water, 0.5 mL of Folin-Ciocalteau reagent and sodium carbonate, and held at dark condition for 35 min. By using a spectrophotometer, the absorbance of the reacting mixture was measured at 765 nm against a blank. The measurement was repeated 3 times and the average was recorded. Gallic acid at various concentrations of 0.01 to 0.07 mg/mL was prepared by dissolving it in methanol. The quantification was performed based on a standard curve with gallic acid and the result is expressed as per gram of gallic acid equivalent (GAE).

Determination of Antioxidant Activity by 2, 2-diphenil-1-picrylhydrzyl (DPPH) assay

The antioxidant activity of the dragon fruit (*H. polyrhizus*) extract was evaluated using DPPH (2, 2-diphenil-1-picrylhydrzyl) free radicals scavenging assay according to Prabowo et al. (2019). The mixture was allowed to stand in a dark room for 30 min. The purple colour solution was formed when dissolving in ethanol as DPPH is a stable free radical. Therefore, the purple colour was bleached as the antioxidant components can scavenge this stable free radical. The absorbance of the solution was measured at 517 nm. Antioxidant activity index (AAI) was calculated according to Formula 1 while the scavenging effect was calculated according to Formula 2 as below:

$$\text{AAI} = \text{final concentration of DPPH in the reaction)/half maximal inhibitory concentration [IC50]} \quad \textbf{Formula 1}$$

$$\text{I\%} = ([\text{Abs}_0 - \text{Abs}_1]/\text{Abs}_0) \times 100 \quad \text{Formula 2}$$

Abs_0 indicated absorbance of the negative control, and Abs_1 is the absorbance with the tested extract at different concentrations.

Degradation Rate

The 10 mL of optimised sample of both thermal and photostability undergo different treatments for heat and light. The degradation rate constants of betacyanin was analysed based on Formulas 3 and 4 below.

$$w(t) = wo \times exp(-kt) \quad \textbf{Formula 3}$$

$$ln\ (\ w(t)\ wo\ ) = -kt \quad \textbf{Formula 4}$$

The degradation rate of the optimised betacyanin and the established commercialised betacyanin was compared using 10 mL of optimised thermal and photostability betacyanin sample extract and 10 mL of the commercialised extract.

RESULTS AND DISCUSSION

Thermal Stability

Temperature is one of the factors that affect betacyanin stability during processing and storage because betacyanins are heat sensitive pigments. In this study, the interactions between temperature, pH, and ascorbic acid were determined using RSM and the optimum condition of the thermal stability was determined. The second-order polynomial derived from the CCD design was follow as Formula 5 below.

$$R = 2.33 + 2.61A - 0.1442B + 0.4575C - 0.3209AB - 0.6874AC + 0.1774BC - 0.8416A\ 2 + 0.6065C\ 2 + 0.7889C^2$$ **Formula 5**

Where A is temperature, B is pH, and C is ascorbic acid.

The value of the coefficient of determination (R^2) and the adjusted coefficient determination (Adjusted R^2) were found to be high, which were 0.9533 and 0.8128 respectively, indicating a satisfactory correlation between independent variables and the response. The model that was developed suggested quadratic model and significant due to the F-value and p-value 64.79 and <0.0001 respectively as shown in Table 2. The f-value was obtained when an ANOVA test was run to determine the means between the populations that are significantly different. Besides, the p-value can also be determined. The F-value must be used in combination with a p-value to evaluate the result significantly with a non-significant lack of fit, which verifies that the model is accurate. The quadratic polynomial model can be expressed by the response after employing multiple regression of actual.

Table 2. Analysis of variance (ANOVA) for thermal stability

Source of variation	Sum of squares	Df	Mean square	F-value	p-value
Model	67.27	6	11.21	64.79	<0.0001
A-light exposure	40.88	1	40.88	236.24	<0.0001
B-pH	1.38	1	1.38	7.99	0.0180
C-Ascorbic acid	1.66	1	1.66	9.59	0.0113
AB	0.9112	1	0.9112	5.27	0.0447
AC	13.98	1	13.98	80.80	<0.0001
BC	4.85	1	4.85	28.04	0.0003
A^2	1.73	10	0.1730		
B^2	1.56	8	0.1954	2.33	0.3344
C^2	0.1674	2	0.0837		
Residual	72.70	17			
Lack of Fit	67.27	6	11.21	64.79	<0.0001
Pure Error	40.88	1	40.88	236.24	<0.0001
Cor Total	1.38	1	1.38	7.99	0.0180

A is temperature, B is pH, and C is ascorbic acid.

Fig. 1 shows that when the temperature is 55 °C with pH 5 and the addition of 0.8 g/mL ascorbic acid, the lowest total betacyanin reduction was found. This is because betacyanin is heat heat-sensitive pigment because as the temperature increases the total betacyanin reduction also increases. Based on the studies carried by Wong and Siow (2015), the temperature 65°C, pH 4 and 0.2 g/mL addition of ascorbic acid have highest betacyanin content. Meanwhile when the temperature of 80°C with pH 5 but without the addition of ascorbic the highest reduction of total betacyanin was observed. From this situation, it can be indicated that the higher the temperature, the higher the reduction of total betacyanin content. The red stationary point indicates the existence of the optimum condition of this model. Temperature is regarded as the most crucial factor governing betalain stability and even though being heat liable pigments, pigment lose stability are elevated temperatures (Herbach et al., 2016). Betacyanin solution is stable and in red colour under neutral and weakly acidic conditions, while unstable under alkaline conditions. The alkaline condition causes aldimine bond hydrolysis while

acidification induces recondensation of yellow betalamic acid with the amine group of addition residue (Schwartz and Elbe, 1983). The supplement of antioxidants like ascorbic acid showed promising results in stabilising red dragon fruit betacyanin with supplementation of 1% of ascorbic acid reported to delay the betacyanin reduction during thermal treatment of dragon fruit (Wong and Siow, 2015). According to Fig. 1, there is an increase in betacyanin degradation after red point. This is due to the excessive addition of ascorbic acid to betacyanin leads to the degradation of ascorbic acid because of chemical transformations that related to the participation of enzymes that become active when the temperature increase (Ajibola et al., 2009).

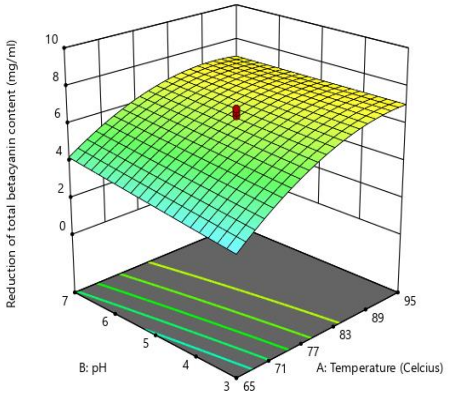

Fig. 1. Three-dimensional plot as A: Temperature; B: pH and C: Ascorbic acid

The experimental and predicted value was compared to determine the validity of the model. The percentage of verification of the model was calculated for the optimised response. The actual results were compared to the predicted value by measuring the residual standard error (RSE) percentage, where the RSE percentage need to be below 10%. As shown in Table 3, the minimum reduction of total betacyanin is 5.591 mg/mL was optimised at 77.428 °C, pH 3 and 1.0 g/mL addition of ascorbic acid. The RSE percentage of the optimum condition was below 10% and represented the model was in good concurrence.

Table 3. Optimum condition for thermal stability

			Reduction of total betacyanin			
Temperature	pH	Ascorbic acid	Actual	Predicted	RSE	Desirability
77.428°C	3.00	1.00	5.913	5.591	5.759	0.918

Photostability

Betacyanin is light sensitive pigment and tends to degrade when exposed to light upon the storage (El-Ashry et al., 2020). In this study, the interactions between temperature, pH, and ascorbic acid were determined using RSM and the optimum condition of thermal stability was determined. The second-order polynomial derived from the CCD design was followed as Formula 4 below.

$$R = 2.33 + 2.61A - 0.1442B + 0.4575C - 0.3209AB - 0.6874AC + 0.1774BC - 0.8416A\ 2 + 0.6065C\ 2 + 0.7889C^2$$ **Formula 4**

Where A is light exposure (hour), B is pH, and C is ascorbic acid.

Quadratic model was suggested as the significant after ANOVA and final regression equation for obtaining a reduction of total betacyanin content at parameter light exposure, pH, and ascorbic acid. The values of the coefficient of determination (R^2) and the adjusted coefficient determination (Adjusted R^2) were found high which were 0.9655 and 0.8504 respectively indicating a satisfactory correlation between independent variables

and the response. The model that was developed suggested a quadratic model and significant due to the F-value and p-value 50.80 and <0.0001 respectively, as shown in Table 4.

Table 4. Analysis of variance (ANOVA) for photostability

Source of variation	Sum of squares	Df	Mean square	F-value	p-value
Model	117.56	9	13.06	50.80	<0.0001
A-light exposure	93.37	1	93.37	363.11	<0.0001
B-pH	0.2840	1	0.2840	1.10	0.3282
C-Ascorbic acid	2.33	1	2.33	9.06	0.0197
AB	0.8237	1	0.8237	3.20	0.1166
AC	3.78	1	3.78	14.70	0.0064
BC	0.2517	1	0.2517	0.9788	0.3554
A^2	9.14	1	9.14	35.56	0.0006
B^2	4.75	1	4.75	18.47	0.0036
C^2	5.08	1	5.08	19.75	0.0030
Residual	1.80	7	0.2571		
Lack of Fit	1.66	5	0.3315	4.65	0.1865
Pure Error	0.1426	2	0.0713		
Cor Total	123.75	17			

From the Fig. 2, the graph shows that when betacyanin undergoes light exposure the reduction of total betacyanin is increased because betacyanin is light sensitive pigment. When the no light exposure to the betacyanin pigment with pH 5 and 0.8 g/mL ascorbic acid, the lowest total betacyanin reduction was found at 0.367 mg/mL. Meanwhile, when the light exposure 70 hour with pH 3 with 0.1 % the addition of ascorbic the highest reduction of total betacyanin was observed (8.456 mg/ml). From this situation, it can be indicated that the longer the exposure of betacyanin to light, the higher the reduction of total betacyanin content. The same finding was observed by El-Ashry et al., (2020) where the total betacyanin content decreased over time when exposed to the light. The red stationary point indicated the existence of the optimum condition of this model. Based on Torres et al., (2020) higher pigment retention was observed when less exposure to light. The effect of light, UV, and visible light towards betacyanin stability induced by the excitation of electrons of betalain chromophore to more energetic state that causing higher reactivity or lower activation energy of the molecule (Hendry & Houghton, 1996). The same observation was observed by Wong and Siow (2015) where at this pH 5 the betacyanin content was significantly higher as compared to pH 7.

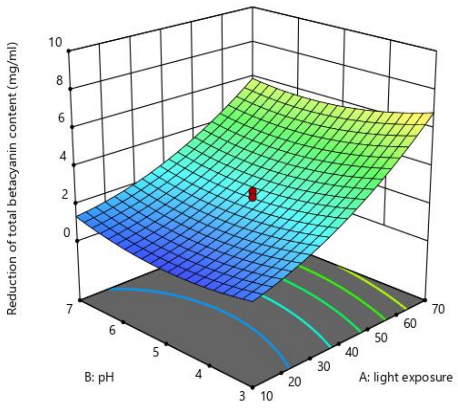

Fig. 2. Three-dimensional plot as A: Light exposure; B: pH and C: Ascorbic acid

Table 5 shows that the minimum reduction of total betacyanin is 2.697 mg/mL was optimised at 44.726 h, pH 5.391 and 0.629 g/mL ascorbic acid. The RSE percentage of the optimum condition was below 10% and represented the model was in good concurrence.

Table 5. Optimum condition for photostability

			Reduction of total betacyanin			
Temperature	pH	Ascorbic acid	Actual	Predicted	RSE	Desirability
44.726	5.391	0.629	2.750	2.697	1.965	0.959

Chemical Analysis

Total phenolic content of H. polyrhizus flesh

The total phenolic content was determined using Folin-Ciocalteau method expressed as gallic acid equivalents. Thermal betacyanin have lower value as compared to the photo sample of betacyanin with 13.607 mg GAE/mL and 18.071mg GAE/mL respectively. According to Abd Manan et al., (2019), the total phenolic content is 32.9 ± 0.92 mg of GAE in 100 mL of fruit juice that higher compared to the current study. The difference in total phenolic content of the sample could be due to differences in sample preparation as in this current study the betacyanin in *H. polyrhizus* undergo optimization while the sample from the past study only undergo normal experimental procedure.

Antioxidant activity of H. polyrhizus flesh

DPPH scavenging activity of both thermal and photo of the optimised sample that compared with ascorbic acid as a positive control. IC_{50} for thermal sample is significantly higher than the photo sample with 546.549 μg/mL and 519.05 μg/mL, respectively. The antioxidant activity of betacyanin in *H. polyrhizus* reported by Prabowo et al., (2019) was 10,012.494 mg/mL. So, from the previous study and this current study proof that (*H. polyrhizus*) is a good source of antioxidant.

Degradation rate of H. polyrhizus flesh

The degradation kinetics of betacyanin for thermal and photostability samples was followed first-order kinetic model (Chew et al., 2019). The degradation rate of the optimised extracted betacyanin is compared to commercial established betacyanin. From the Table 6, the degradation rate of the extracted betacyanin is higher than the commercial sample with 0.0368 min-1 and 0.0084 min-1 respectively.

Table 6. Degradation rate (k) and correlation factor (R2)

Sample	Sample	Degradation rate, (min-1)	Regression coefficient (R2)
Thermal stability	Extracted	0.0368	0.9229
	Commercial	0.0084	0.9661
Photostability	Extracted	0.0200	0.9637
	Commercial	0.0107	0.9096

Table 6 shows that extracted betacyanin degraded faster than commercial betacyanin. Temperature causes degradation of betacyanin when exposed to processing temperature and degradation can be minimised by keeping betacyanin from processing time during heating. Betacyanin degraded during heat processing due to the isomerisation, decarboxylation or cleavage by heat resulting in reduction of total betacyanin content. The same observation was found by Bolivar and Cevallos-Casals (2003), where commercial red grape colourants have slightly higher stability than purple corn. This is due to the higher initial polymeric colour of commercial colourants that causes by more severe processing conditions and previous longer storage. Fresh extracted has most of its anthocyanin in the monomeric form and upon processing, the polymerising started that lead to the increase in polymeric material. In this current study, the temperature dependence of betacyanin degradation is consistent with other reports on red dragon fruit betalain degradation (Kunnika and Pranee, 2011).

In the present of light, betacyanin degraded due to light emission to the pigments. Higher pigment retention was observed when less light exposure (Torres et al., 2020). Light also has a detrimental effect on betacyanin

stability. Due to the photostability of these pigments it is advisable to keep these pigments in dark or low lighting conditions to minimise pigments destruction. Absorption of light in the ultraviolet and visible region increases the betacyanin reactivity towards oxygen (Herbach et al., 2006). The degradation rate of both extracted thermal and photo optimised sample of betacyanin was higher compared to the commercial established betacyanin because of the different physical forms of the sample. Extracted betacyanin was in liquid form while commercially established betacyanin was in powder form. The powder form of commercial betacyanin is more stable due to its low water activity and low biochemical reaction (Muhammad et al., 2015). The commercial may apply several treatments to improve the stability of the product. This includes encapsulation. Betacyanin stability can be prevented and improved by several methods such as preservation in darkness, the addition of stabilisers, pH control, minimum heat treatment and microencapsulation to coat and isolate the pigment from external environment to increase its shelf life (Fernández-López et al., 2020).

Encapsulation of betacyanin by using maltodextrin as wall material with the combination of gum Arabic and red dragon fruit pectin according to Rodriguez et al. (2015) give high encapsulation efficiency. This encapsulation can maintain and hold core materials inside the microparticles attributed to the capability of biopolymeric wall materials to interact with betacyanin. The studies by Handayani et al. (2018) also mentioned that encapsulation of betacyanin with maltodextrin increased the encapsulation of betacyanin in dragon fruit.

CONCLUSION

RSM is a useful tool to identify the optimum condition for betacyanin in *H. polyrhizus*. By using the central composite design, the optimised value of thermal and photo optimised sample of betacyanin extract were 77.428°C, pH 3 and 1.000 g/mL of ascorbic acid and 44.726 hours, pH 5.391 and 0.629 g/mL ascorbic acid respectively. The low temperature of thermal treatment is recommended with the intention to pasteurise and retain betacyanin dyes in *Hylocereus polyrhizus*. By subjecting the product to low temperature, the heating time can be increased to kill the microorganisms and retain the colour. Betacyanin is a light-sensitive compound. When displayed on shelves with light exposure, absorption of light will excite the electrons of the betalains chromophore and increase its reactivity, which will result in betacyanin degradation. The degradation rate of optimised sample of betacyanin was compared with the commercially established betacyanin. The degradation rate of commercially established betacyanin was found to be degraded lower than optimised sample of betacyanin. Betacyanin also has a high value of TPC and antioxidant for both optimised samples for thermal and photo sample.

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