Determining Pigment Extraction Efficiency and Pigment Stability of Dragon Fruit (*Hylocereus polyrhizus*)

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**Abstract:** Pigment extracted from the pulp of *Hylocereus polyrhizus* showed a difference in colour when subjected to room temperature extraction and 100°C extraction. The room temperature extraction yielded red-purple coloured pigments while the 100°C extraction yielded scarlet red coloured pigments. Experiments were carried out to investigate the best water:weight ratio and optimum temperature to obtain highest pigment concentration, best extraction method and best storing condition. Best water:weight ratio was 1:1, best temperature observed was 100°C, best extraction method was by using juice concentrate rather than water extraction and the best storing condition was storage in -20°C. Both extraction methods exhibited stable pH reading after one week of storage. Nevertheless, the best method to obtain high pigment concentration is by using juice concentrate which is at least two times more concentrated than samples extracted with water.

**Key words:** Betacyanin, betalain, cactaceae, dye, food colouring, natural colourant, pulp

**INTRODUCTION**

Fruits of the genus *Hylocereus* (Berger) Briton and Rose originated from Latin America and are known as red pitaya belonging to the Cactaceae family (Stintzing et al., 2002). This vine-like epiphytic cacti is also cultivated in Vietnam, Malaysia, Taiwan, China, Okinawa, Israel and Southern China. Producing a deep purple-coloured flesh comparable to red beet or amaranth, fruits from *Hylocereus polyrhizus* are highly appealing in the European and United States market.

Food colouring is a common pre-requisite to compensate for process-related losses to improve overall appearance of food and this factor is important to meet customers’ expectations. Natural food dye is gaining popularity especially in the food and beverage sectors due to strong demand for more natural products by health-conscious consumers (Herbach et al., 2007). Some examples of natural food colorants which have already been used in the food industry include anthocyanin, curcumin, beetroot red, caramel, lycopene, paprika extract and chlorophyll. In addition, natural colours and pigment from fruits and vegetables may contribute additional nutritional value to food coloured as observed in cactus fruits (Mohammer et al., 2005).

Dragon fruit is one of the new focus for the next source of food dye is because it is rich in betalains which are the similar array of colour pigments found in beetroot. Beetroot has been the most important betalain source for natural red colouring and is mainly composed of the red-purple betanin and the C15-isomer isobetanin. However, there is a demand for alternative compounds because of the unfavourable earthy flavour caused by geosmin and pyrazine derivatives, as well as high nitrate concentrations associated with the formation of carcinogenic nitrosoamines (Esquivel et al., 2007). Hence, fruits from the Cactaceae family have been suggested as a promising betalain source being devoid of the mentioned drawbacks.

Betalains are nitrogenous vacuolar pigments and important chemotaxonomical markers found in 13 families within the plant kingdom and in some members of the Basidiomycetes (Stintzing et al., 2004). Betalains have never been found co-existing with the widely distributed plant pigment anthocyanin which explains their roles as markers. Some advantages that betalains possess over anthocyanins include, being more water soluble, a tinctorial strength up to three times higher than anthocyanins and a wider pH stability range from pH 3 to 7 making it suitable for application in a broad palette of low-acid and neutral food (Stintzing and Carle, 2007).

Betalains are divided into the red-purple betacyanins and yellow-orange betaxanthins which comprise about 55 different structures and promise a great variation of colour array to the food industry. In dragon fruit alone, there are at least seven known betalain namely; betamin, isobetanin,
phylllocaetic, isophylllocaetic, betamidin, isobetamidin and 
bougainvillea-r-I (Stintzing et al., 2002) all of which have 
identical absorption spectra (λ_max) that contribute to the 
deep-purple colour observed in the fruit pulp.

This study is aimed at exploring the possibilities of water 
extration method and heat to obtain significant 
pigment concentration and to test the stability of samples 
stored at different temperatures.

**MATERIALS AND METHODS**

**Plant material:** Dragon fruits were obtained from 
Multi Rich farm in Nilai, Negeri Sembilan, Malaysia on 
21 February 2008. All fruits were freshly harvested and 
transported to the Postharvest Biotechnology laboratory 
in the University of Malaya for experiment. Fruits were 
treated with Benomyl 0.05% and air dried overnight. Fruit 
pulp was cut into small cubes, frozen under liquid 
nitrogen and stored in -20°C until used.

**Sample measurements:** Absorbance for samples were 
measured at 538 nm using a spectrophotometer 
(Pharmacia, Ultrospec II) to determine total betalain 
concentration while pH was measured using a Hanna pH 
meter. All extracts were filtered using Whatman paper 
No. 1 (9 cm) to remove the pulp and obtain the aliquot. All 
experiments were carried out in triplicates.

**Efficiency of water volume to extract pigment:** Five grams 
of pulp were immersed in 5, 10, 15 and 25 mL of Sterilized 
Distilled Water (SDW) for 10 min to determine which ratio 
of weight: volume yields highest pigment concentration. 
The pH and absorbance of the aliquots were measured. 
The best weight: volume ratio was then used for 
subsequent experiments.

**Efficiency of temperature to extract pigment:** Five grams 
of pulp were immersed in 5 mL SDW and pigments were 
extracted at room temperature (RT), 40, 60, 80 and 100°C 
for 10 min to determine which temperature yields highest 
pigment concentration. The pH and absorbance of the 
aliquots were measured. The best temperature to obtain 
highest pigment concentration was then used for 
subsequent experiments.

**Stability of pigments extracted with water**

**Sample preparation:** Five grams of pulp were immersed in 
5 mL of SDW and pigments were extracted at 100°C using 
a waterbath for 10 min. All solutions were filtered to 
remove the pulp. The pH and absorbance of the aliquots 
were measured before used for experiments.

**Stability test samples:** Five milliliter of aliquots were 
stored at RT, 4 and -20°C in test tubes. The test tubes that 
contained the samples for dark storage were wrapped with 
aluminum foil. All samples were stored for one week and 
then taken out for further analysis.

**Stability of pigments from juice concentrate**

**Sample preparation:** Approximately 500 g of fruit pulp 
were sieved to obtain concentrated juice. Five milliliter of 
juice was poured into test tubes and extracted at 100°C for 
10 min while another 5 mL of juice was poured into test 
tube at room temperature as control. The pH and 
absorbance at 538 nm of each solution were determined 
before used for experiments.

**Stability test samples:** Five milliliter of juice were stored 
at RT, 4 and -20°C in test tubes. The test tubes that 
contained the samples for dark storage were wrapped with 
aluminum foil. All samples were stored for one week and 
then taken out for further analysis.

**Determination of total betalain concentration in samples:** 
Absorbance values of samples were measured using a 
spectrophotometer (Pharmacia, Ultrospec II) at 538 nm 
against a blank of SDW. The absorbance obtained was 
then used to calculate the total betalain concentration 
using the following formula (Herbach et al., 2007).

\[
BC \text{ (mg L}^{-1}\text{)} = \frac{A \times MW \times 1000 \times DF}{c \times l}
\]

- \(A\) = Absorbance
- \(DF\) = Dilution factor
- \(MW\) = Molecular weight of betalin
- \(c\) = Molar extinction coefficients
  - 550 g mol\(^{-1}\)
- \(l\) = Path length of cuvette = 1 cm

**RESULTS**

**Efficiency of water volume to extract pigment:** Figure 1 
shows that the pH for 5 g of pulp extracted in different 
volumes of SDW did not show any significant differences 
while Fig. 2 shows the total betalain concentration for the 
same solution. The total betalain concentration obtained 
from 5 g of pulp in different volumes of SDW was 25.5, 
25.3, 24.9 and 24.2 mg L\(^{-1}\), respectively and the highest 
yield would be samples which had 5 g of pulp extracted in 
5 mL of SDW.
Fig. 1: pH of 5 g pulp in extracted with different volumes of SDW. The pH for samples in different volumes of SDW did not show any significant changes.

Fig. 2: Total betalain concentration of 5 g pulp in different volumes of SDW. The highest yield of pigments was obtained by extracting 5 g of pulp in 5 mL of SDW.

Efficiency of temperature to extract pigment: Figure 3 shows the pH of 10 g pulp extracted in 10 mL of SDW at different temperatures and the results showed that pH did not vary much. Figure 4 shows that the total betalain concentration obtained after the heat treatment at RT, 40, 60, 80 and 100°C was 26.2, 25.6, 26.1, 25.7 and 26.2 mg L⁻¹, respectively. The highest yield was samples that were extracted at RT and 100°C.

Stability of pigments extracted with water
Samples extracted at room temperature: Figure 5 shows the pH of samples at day 0 and 7 after being stored in different temperatures and conditions. The pH did not exhibit any significant change after 1 week of storage. Figure 6 shows the total betalain concentration of the same samples and there was a significant increase after one week of storage indicating that pigments under went structural changes or degradation. The highest increase was observed in samples that were stored at 4°C in the dark, from 26.20 mg L⁻¹ at day 0 to 94.78 mg L⁻¹ at day 7.

Fig. 3: pH of 10 g pulp in 10 mL of SDW at different temperatures. There was no significant changes in pH value after samples were subjected to different temperatures for pigment extraction.

Fig. 4: Total betalain concentration of 10 g pulp in 10 mL of SDW at different temperature. There was no significant difference in all samples extracted at different temperatures but the highest pigment yield was obtained in samples extracted at room temperature and 100°C.

Fig. 5: pH changes in samples extracted with SDW at room temperature stored in different conditions and temperatures. The pH value of samples did not show any significant change after one week of storage.
Fig. 6: Total betalain concentration in samples extracted at room temperature and stored in different conditions and temperatures. It was observed that there was a significant increase of total betalain content after one week of storage in all samples.

Fig. 8: Total betalain concentration of samples extracted at 100°C and stored in different conditions and temperature. It was observed that there was a significant increase of total betalain content after 1 week of storage in all samples.

Fig. 7: pH of samples extracted at 100°C and stored in different conditions and temperatures. It was observed that all samples exhibited a slight increase in pH value after one week of storage while the lowest increase, thus the most stable, was observed in samples that were stored at -20°C exposed to light, from 26.32 mg L⁻¹ at day 0 to 63.12 mg L⁻¹ at day 7.

Fig. 9: pH changes of juice concentrate samples stored at different temperatures and conditions. All samples showed a slight increase after one week of storage except for samples kept at room temperature in the dark that showed a slight decrease.

Samples extracted at 100°C: The pH for all samples exhibited a slight increase at day 7. The highest increase of pH was observed in samples stored at room temperature exposed to light, from pH 5.25 at day 0 to pH 6.02 at day 7. The lowest increase, thus most stable, was observed in samples that were stored at -20°C in the dark, from pH 5.34 at day 0 to pH 5.4 at day 7 (Fig. 7). Figure 8 shows the total betalain concentration of the same samples and there was a significant increase after 1 week of storage indicating that pigments under went structural changes or degradation happened. The highest increase of total betalain concentration was observed in samples that were stored at room temperature exposed to light, from 25.60 mg L⁻¹ at day 0 to 129 mg L⁻¹ at day 7. The lowest increase, thus most stable, was observed in samples that were stored at room temperature in the dark from 25.80 mg L⁻¹ at day 0 to 67.76 mg L⁻¹ at day 7.

Stability of pigments from juice concentrate

Juice concentrate extracted at room temperature: Figure 9 shows the pH of juice concentrate samples stored in different temperatures and conditions. All samples except samples kept at room temperature in the dark, showed a slight increase in their pH value after one week of storage. Figure 10 shows the total betalain concentration in the same samples in which all samples showed a decrease in total betalain concentration after 1 week storage except for samples kept at -20°C which showed an increase in total betalain concentration. The highest decrease was observed in samples stored at room temperature exposed to light, from 246 mg L⁻¹ at day 0 to 67.76 mg L⁻¹ at day 7.
was observed in samples that were stored at 4°C exposed to light, from pH 4.63 at day 0 to pH 5.07 at day 7 while the lowest increase was observed in samples that were stored at room temperature exposed to light, from pH 4.66 at day 0 to pH 4.78 at day 7 (Fig. 11). The results showed that there was a general decrease in total betalain concentration in samples after one week of storage. The highest decrease was observed in samples stored at -20°C in the dark, from 247.75 mg L⁻¹ at day 0 to 163.45 mg L⁻¹ at day 7 while the lowest decrease was observed in samples that were stored at 4°C exposed to light, from 246.64 mg L⁻¹ at day 0 to 215.58 mg L⁻¹ at day 7 (Fig. 12).

**DISCUSSION**

**Efficiency of water volume to extract pigment:** The best water volume to be used in extracting pigment from 5 g of pulp is 5 mL of SDW. It is highly possible that any extraction procedure to obtain best yield of pigments from dragon fruit pulp is by using the ratio of 1:1 (weight: volume).

**Efficiency of temperature to extract pigment:** As betacyanins undergo thermal treatment, it is known that the betalains (major pigment class in dragon fruit) will experience degradation and fluctuating chromatic stability (Herbach et al., 2006a). The betalains will be subjected to processes like isomerization, deglycosylation, decarboxylation, hydrolysis and other processes. The specific main pigment known in dragon fruit which is betanin gives the red-purple colour. As indicated in the result, samples extracted at 100°C also gives a comparable high yield of betalain content at 538 nm to samples subjected to room temperature. It is possible that the occurring pigment which is scarlet red after 100°C thermal treatment is isobetanin, the isomer of betanin.

**Stability of pigments extracted with water:** All samples extracted at RT and 100°C showed a significant increase in total betalain concentration after one week of storage in the different temperatures. The increase in samples extracted at 100°C can be explained by the fact that betanin has the ability to regenerate by recondensation of hydrolys products associated with a colour regain (Stintzing and Carle, 2007). It is also possible that when betacyanins are extracted with water, it is drawn out from its protective matrices where the condition in the pulp is the stable environment for betacyanins.

Thus, the main pigment may undergo multiple structural adjustment/regeneration to stabilize itself in the water as oppose to pigments used directly from the juice concentrate without any alteration of physical condition.
This regeneration is encouraged when pH value is close to pH 6 with the presence of the basic building blocks of the betacyanins cyclo-DOPA ring and betalamic acid, which forms the betacyanin chromophores.

The results show that betacyanins have great pigment retention ability even after being heated and stored in adverse conditions and exposed to illumination. Samples which exhibited minimal pH change after one week of storage were samples extracted at room temperature and kept in 4°C, exposed to light while samples which exhibited minimal total betalain change were samples extracted at room temperature, kept at -20°C and exposed to light.

Studies on the stability and the regeneration of betacyanins in dragon fruit are still at an early stage because this is a relatively new crop being focused on as a natural food dye source. A noteworthy behaviour of betalain (the major pigment in *Hylocereus polyrhizus*) that should be taken into consideration in future experiments is that, it undergoes multiple processes like decarboxylation, deglycosylation, hydrolysis, isomerization, dehydrogenation and others when it is subjected to these factors: water activity, pH change, antioxidants present, chelating agents, temperature (heat), illumination and oxygen/nitrogen atmosphere (Herbach et al., 2006b). The structural alterations that occur will give a different pigment configuration but all these betacyanins still give a purple-red colour which is detected at 538 nm using the spectrophotometry method.

From the results obtained, it was observed that samples extracted at room temperature showed minimal pH and total betalain content changes as compared to samples extracted at 100°C. This shows that pigments subjected to heat during extraction exhibit lower pigment stability and support the findings that heat is an important stability factor as previously mentioned. Samples stored at -20°C in both extraction methods showed minimal change compared to other samples stored at room temperature and 4°C which suggests that lower temperature stabilizes the pigments more effectively.

**CONCLUSION**

The wide array of betacyanins present in *Hylocereus polyrhizus* provides an avenue to obtain a new natural food colourant. The results in this study showed that the best weight: volume ratio to extract pigments with water is 1:1 and the best temperature to use for high pigment yield is 100°C. The pH and pigment retention capacity observed in this study revealed that betacyanin have high tolerance towards factors such as temperature and light which is most important in food colouring stability. Other than that, the results indicated that the betacyanins have the ability to regenerate under suitable conditions which supports earlier findings mentioned earlier. The most stable condition for pigment storage observed in this study where there was least change in pH and pigment concentration, was samples stored -20°C. Visible colour changes that was observed when samples were subjected to heat suggests that there is a possibility that structural conformation occurred but pigment concentration did not. Overall, this study can conclude that water extraction and heat are viable methods to obtain high concentration of betacyanins and these pigments have a great tolerance towards factors that are important when it comes to food colouring, water extraction could be more economical and heat may provide an alternative colour for a natural dye. Further studies and experiment are needed to ascertain and confirm these initial findings. Thus, the potentials and promising findings so far on *Hylocereus polyrhizus* makes the crop a new valuable source of water-soluble and natural dye for health conscious consumers along with the food additive industry.
REFERENCES


