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Study of Optimal Temperature, pH and Stability of Dragon Fruit (*Hylocereus polyrhizus*) Peel for Use as Potential Natural Colorant

K.V. Harivaindaran, O.P.S. Rebecca and S. Chandran
Institute of Biological Sciences, Faculty of Science, University of Malaysia,
50603 Kuala Lumpur, Malaysia

Abstract: The peel of *Hylocereus polyrhizus* is often regarded as a waste hence this study was aimed at exploring the feasibility of using the peel as a natural colorant using simple water extraction method. Samples were subjected to a series of temperatures: Room temperature (RT), 50, 80 and 100°C; varied length of heating time from 1, 2, 3, 4, 5 and 10 min and a varied range of pH using 1 M of citric acid solution. The best condition to obtain highest betacyanin content was heating samples at 100°C for 5 min in a pH 5 citric acid solution. The next part of this study involved the stability test of the pigments obtained through the best method determined earlier. The pigments were dried and resuspended in distilled water. The samples were then exposed to light to monitor pigment changes. Initial resuspension of the dried pigments yielded a comparable high content of betacyanins to its juice counterpart. The results showed that resuspended pigments had high pigment retention and were stable up to 7 days. These initial findings must be further studied in more controlled conditions to understand the stability of betacyanin. Nevertheless, the results show that betacyanin obtained from the peel of dragon fruit has a high potential to be used as a natural dye.

Key words: Betalain, betacyanin, cactaceae, colourant, dye, food colouring, peel

INTRODUCTION

Hylocereus polyrhizus or more commonly known as the pitaya or the dragon fruit is a member of the Cactaceae family from the genus *Hylocereus*. The flesh of the fruit is red-purple in colour when ripened with minute black seeds interspersed and has gained a growing interest for cultivation in Malaysia (Hoa *et al*., 2006). This fruit has gained much interest in the society because of its exotic features, attractive colours, nutritional value and pleasant taste (Le Bellec *et al*., 2006).

Natural dyes are colorants obtained from biological matter through mechanical retention, covalent chemical bonds formation or complexes with salts or metal formations, physical absorption, or by solutions. Natural colorants have a vast economic significant because the dye trade has a world market worth £2.5 billion/year.

Betalains were thought to be flavonoids but they have been found to contain nitrogen and do not change colour reversibly in the same way as anthocyanins do to pH. Betalains were first extracted from the red beet (*Beta vulgaris*) and is used mainly for food colouring. The extract contains red and yellow pigments, namely the betacyanins and betaxanthins, respectively. Betacyanin is the major component (95%) of the red pigments in the extract. Never occurring together in the same source, the betalains are found in place of the anthocyanin group of pigments in plants of the Caryophyllales Family (Strack *et al*., 2003; Kimler *et al*., 1971; Cai *et al*., 2005). However, there is a demand for alternative source other that the red beet because of the unfavourable earthy flavour caused by geosmin and pyrazine derivatives, as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines (Esquivel *et al*., 2007). Dragon fruit is one of the new focuses for the next source of red dye because it is rich in betalains which are the similar array of colour pigments found in beetroot and devoid of the mentioned drawbacks.

The objective of this research is to study the colorant of the dragon fruit peel at various temperatures, pH and length of time to heat exposure to determine the optimal condition for extracting the dye as natural colorant. The extraction method employed the use of distilled water, since betacyanin peel is water soluble. Stability of the colorant towards light was studied, spectrophotometric and pH analysis was carried out for all samples.

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

Corresponding Author: Chandran Somasundram, Institute of Biological Sciences, Faculty of Science, University of Malaysia, 50603 Kuala Lumpur, Malaysia Tel: +603-7967 4423 Fax: +603-7967 4178

transported to the postharvest laboratory in 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 all 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 into test tubes using mira cloth to remove the peel and obtain the aliquot. Resulting aliquots in each experiment was allowed to cool before taking spectrophotometric measurements. All experiments were carried out in triplicates.

Determination of total betacyanin content in samples: The absorbance readings obtained was used to calculate the total betalain concentration for each sample using the following formula (Herbach *et al*., 2007):

$$SC\ (mg\ L^{-1}) = \frac{A \times MW \times 1000 \times DF}{\epsilon \times \iota}$$

Where:

A = Absorbance
DF = Dilution factor
MW = Molecular weight of betanin
= 550 g mol^{-1}
ϵ = Molar extinction coefficients
= 60,000 L/mol cm in H_2O
ι = Path length of cuvette = 1 cm

Determination of optimal temperature: Ten gram of dragon fruit peel was immersed into 30 mL of distilled water and the dye was extracted at RT for 5 min. The pH and absorbance of the aliquots were measured. The experiment was repeated at 50, 80 and 100°C. The best result was subsequently used in the next experiment.

Determination of optimal length of time to heat exposure: Ten gram of peel was added into a beaker containing 30 mL of boiling distilled water. The solution was left for 1 min. The pH and absorbance of the aliquots were measured. The experiment was repeated at 2, 3, 4, 5 and 10 min at the same temperature. The best result was subsequently used in the next experiment.

Determination of optimal pH value: Distilled water at different pH was prepared by adding 1 M of citric acid into distilled water until the desired pH is obtained. Ten gram of peel was added into 30 mL of pH 2 distilled water and heated to 100°C. The extraction was carried out for 5 min while, maintaining the temperature through out. The pH and absorbance of the aliquots were measured. The experiment was repeated using distilled water with different pH: 3, 4, 5 and 6. The best result was subsequently used in the next experiment.

Determination of colour stability: Ten gram of peel was added into 30 mL of pH 5 distilled water and heated to 100°C. The extraction was carried out for 5 minutes while maintaining the temperature through out. The pH and absorbance of the aliquots were measured. A control was prepared with 10 g of peel immersed in 30 mL of distilled water for 5 min. Both aliquots were exposed to light for 12 h each day.

Determination of resuspended colour stability: Extracts were prepared from heating 10 g of peel in 30 mL of pH 5 distilled water heated to 100°C for 5 min. The aliquots were evaporated in the oven overnight to dry. Every 1 g of dried aliquots collected was resuspended in 50 mL water. The pH and absorbance for the samples were taken before and after drying. The control for this experiment was dried extract from 10 g of peel in 30 mL of distilled water at RT for 5 min.

RESULTS

Determination of optimal temperature: Figure 1 shows that the total betacyanin content obtained from 10 g of peel in 30 mL of distilled water at different temperatures were significantly different. The total betacyanin content obtained at RT, 50, 80 and 100°C were 5.34, 10.47, 12.69 and 24.03 mg L^{-1}, respectively. The highest yield of betacyanin content was obtained from sample heated at 100°C.

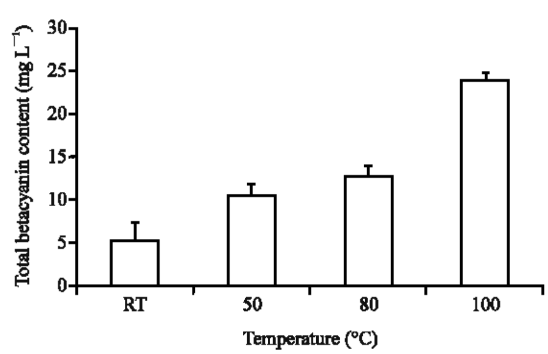

Fig. 1: Total betacyanin content from 10 g peel in 30 mL distilled water at different temperatures

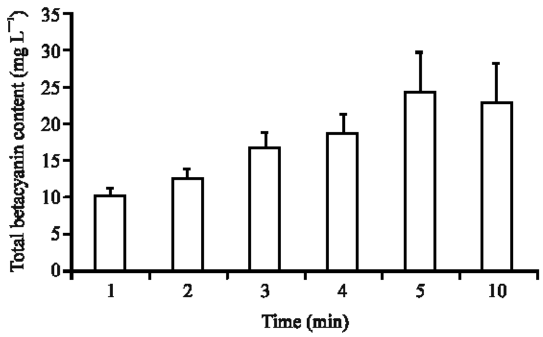

Fig. 2: Total betacyanin content from 10 g peel in 30 mL distilled water at 100°C for different length of time

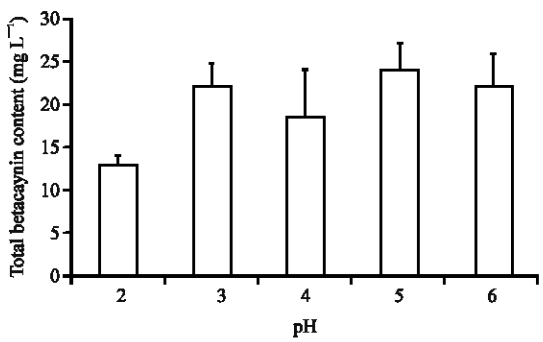

Fig. 3: Total betacyanin content from 10 g peel in 30 mL of different pH distilled water at 100°C for 5 min

Determination of optimal length of time to heat exposure: Figure 2 shows that the total betacyanin content obtained from heating 10 g peel in 30 mL of distilled water at 100°C at 1, 2, 3, 4, 5 and 10 min were significantly different. The total betacyanin content obtained at 1, 2, 3, 4, 5 and 10 min were 10.54, 13.95, 18.37, 19.03, 27.98 and 26.25 mg L^{-1}, respectively. The highest yield of betacyanin content was obtained from samples heated at 100°C for 5 min.

Determination of optimal pH value: Figure 3 shows that the total betacyanin content obtained from heating 10 g peel in 30 mL of distilled water at 100°C for 5 min in different pH were significantly different. The total betacyanin content extracted in 2, 3, 4, 5 and pH 6 distilled water were 12.45, 23.14, 16.79, 25.74 and 22.70 mg L^{-1}, respectively. The highest yield of betacyanin content was obtained from samples heated at 100°C for 5 min in pH 5 of distilled water.

Determination of stability to light: Figure 4 shows the betacyanin content of sample and the control monitored over a period of 14 days. The graph shows that the betacyanin content decreased over time. On day 1, the total betalain content of samples was 22.12 mg L^{-1} while, the control yielded 3.97 mg L^{-1}. At day 14, the betacyanin

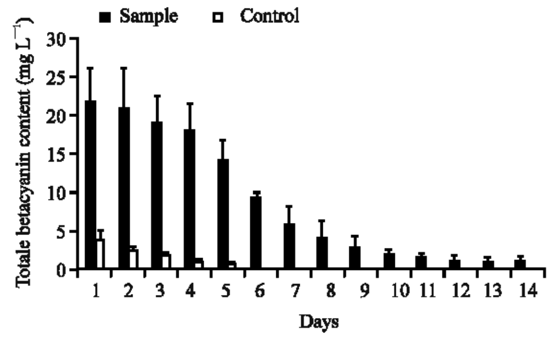

Fig. 4: Total betacyanin content of samples kept in 16 h of light exposure daily for 14 days

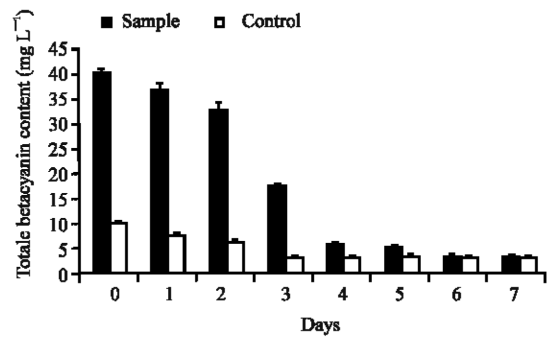

Fig. 5: Total betacyanin content of samples resuspended in distilled water, exposed to light for 7 days

content of samples decreased to only 1.13 mg L^{-1}. The control was terminated at day 6 when absorbance readings were zero indicating that betacyanin was no longer present.

Determination of resuspended colour stability: Figure 5 shows the betacyanin content of sample and the control monitored over a period of 7 days. The graph shows that the betacyanin content decreased over time. On day 1, the total betalain content in samples was 39.97 mg L^{-1} while, the control had 10.12 mg L^{-1}. At day 7, the betacyanin content in samples decreased to only 3.12 mg L^{-1} while, control decreased to 3.03 mg L^{-1}. The sharp decrease observed can be explained by the fact that the dried extract was exposed far longer to both heat and oxygen during the drying process.

DISCUSSION

The optimal temperature, time and pH to obtain highest yield of betacyanin content from dragon fruit peel was heating samples at 100°C, for 5 min with pH 5 distilled water. Heating of water during betacyanin extraction from peel resulted in betacyanins being drawn out from the matrix constituents more effectively. Samples extracted at

100°C gave highest yield despite exposure to extreme heat and this can be explained by the fact that betanin has the ability to regenerate by recondensation of hydrolysis products associated with a colour regain (Stintzing and Carle, 2007). As betacyanins undergo thermal treatment, it is known that the pigments will experience degradation and fluctuating chromatic stability (Herbach *et al*., 2006b). As shown in Fig. 1, samples extracted at 100°C gave a comparable high yield of betacyanin content to samples heated at other temperatures. It is possible that the occurring pigment after 100°C thermal treatment is isobetanin, the isomer of betanin (Herbach *et al*., 2006b). The structural alterations that occur after heat treatment will give a different pigment configuration but all betacyanins still gives a same wavelength colour which is detected at 538 nm using the spectrophotometry method.

The best length of time for heat exposure to obtain high yield of betacyanin content was for 5 min. When samples were heated for 10 min, it yielded a lower betacyanin content as indicated in Fig. 3. This shows that prolonged heating has detrimental effects betacyanin retention and stability. Although, regeneration of the pigment is possible, it becomes less efficient as pigments are exposed to heat in an extreme manner (Herbach *et al*., 2004). The pH determination in this experiment showed that pH 5 is the best pH condition to extract and obtain highest yield of betacyanin content. According to previous studies, betalain pigments favor a pH range of 4 to pH 6 in the presence of oxygen and also under anaerobic conditions (Herbach *et al*., 2006a).

According to Herbach *et al*. (2006b), non-treated pitaya juice is able to regenerate 3% of its pigment while heat-treated juice recorded a 10% regeneration in pigment regeneration. This phenomenon can be associated to the ability of betacyanins to regenerate as long as the basic building blocks are present: cyclo-DOPA ring and betalamic acid, which forms the betacyanin chromophores. When pitaya juice is subjected to heat, the betacyanins will undergo multiple structural adjustments like isomerization, deglycosylation, decarboxylation and hydrolysis (Herbach *et al*., 2006a) to stabilize and regenerate itself as oppose to non-treated juice without any alteration of physical condition. This further reinforces the possibility of heat treated dragon fruit juice for use as potential dye and heat treatment can improve pigment yield.

The stability test in this study showed that betacyanin content decreased upon prolonged light exposure. Earlier studies also showed that betacyanins are light-sensitive pigments and tend to degrade due to light absorption in the visible light and ultra-violet range the betalain molecules (Cai *et al*., 2005; Herbach *et al*., 2006b). This phenomenon leads to an excitation of electrons of the betalain chromophore, causing it to transcend to higher energy states. In this condition the reactivity of the molecules are higher, in other words, the activation energy of the molecules are lowered causing the ready degradation of the pigments.

The dried extract that was resuspended in distilled water showed an intense purple-red aliquot and significantly high content of betacyanin. These results indicate that pigment extracted from dragon fruit peel are highly promising to be sourced as natural dye although the stability pales compared to maintaining the pigments in the form of juice. This can be explained by the fact that the extracts were exposed to further heat in the drying oven overnight. Temperature is regarded as the most crucial factor governing betalain stability and even though being heat liable pigments, pigments lose stability at elevated temperatures (Herbach *et al*., 2006c). It is therefore the resuspended dried extracts would be far less stable even though pigment retention after drying is significant.

CONCLUSION

The flesh and peel from dragon fruit is an avenue where there are wide array of betacyanins to be exploited as natural food dye or colourant. From this study, samples yield highest betacyanin content at 100°C in pH 5 distilled water and heated for 5 min. 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. Stability tests carried out showed gradual degradation but further studies with strictly controlled environment and conditions will ascertain the stability of the pigments. When pigments were dried and resuspended in this study, the resulting aliquot showed comparable pigment retention which is promising to developing a natural dye in powder form which is preferred by consumers. Further studies and experiment are needed to ascertain and confirm these initial findings. Thus, the potentials and promising findings so far on the peel of dragon fruit makes the crop a new valuable source of water-soluble and natural dye for health conscious consumers along with the food additive industry.

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