

## STABILITY OF BETACYANIN PIGMENTS FROM RED PURPLE PITAYA FRUIT (*Hylocereus polyrhizus*) : INFLUENCE OF PH, TEMPERATURE, METAL IONS AND ASCORBIC ACID

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### ABSTRACT

Betacyanin pigments from red-purple pitaya fruit (*Hylocereus polyrhizus*) could be an attractive source of red colourant for food application. This paper presents results on the extraction of betacyanin pigments from pitaya fruits grown locally in Malaysia. Both the flesh of the fruit and its mesocarp were investigated and it was found that the flesh had higher pigment contents compared to its peel component. The concentration of betacyanins expressed as betanin equivalents per 100 g of flesh and peel were  $10.1 \pm 0.6$  mg and  $6.7 \pm 0.2$  mg, respectively when 80% methanol was used. The stability of betacyanin pigments were investigated at different pH, temperature and in presence of different concentrations of metal ions ( $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$ ) and ascorbic acid. The results showed that the pigment was most stable at pH range between 5 and 6. However, it forfeited its stability to the heat induced at elevated temperatures. Metal ions ( $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$ ) proved to be capable of accelerating betacyanin degradation, with  $\text{Cu}^{2+}$  exhibiting the greatest effect. By contrast, supplementation with ascorbic acid could enhance the pigment stability against the detrimental effects caused by pH, temperature and metal ions. Nevertheless, if the concentration of ascorbic acid exceeds 0.7 %, it may change its role from pigment stabilizer to become a pro-oxidant.

Keywords: Betacyanin, pigments, pitaya fruit, *Hylocereus polyrhizus*, ascorbic acid

### INTRODUCTION

Natural colourants from plant sources are receiving growing interest from both food manufacturers and consumers in the continuing replacement of synthetic dyes [1,2]. However, replacing synthetic dyes with natural colorants offers a challenge because the colour and stability of plant pigments are dependent on several factors, including structure and concentration of the pigment, pH, temperature, light intensity, presence of, metallic ions, enzymes, oxygen, ascorbic acid, sugars and their degradation products, among others [3]. Identifying stable aqueous colorant extracts (e.g. fruit and vegetable juices) is attractive because their GRAS (Generally Recognized As Safe) status makes them easily commercialized.

The fruits of *Hylocereus* species, known as red pitaya or pitahaya, which means "the scaly fruit", in Latin America, belong to the cacti family which is native to the tropical forest regions of Mexico and Central and South America. In cacti, the most important fruit pigments are the red-violet betacyanins and the yellow betaxanthins which belong to the betalain pigments [4]. Producing a deep purple-coloured flesh comparable to red beet [5] or amaranth [6], betacyanins from purple pitaya (*H. polyrhizus*) has recently attracted interest as a potential alternative for red- beet. In the present study, betacyanin from purple pitaya (*H. polyrhizus*) was extracted using several solvents. However, the lower stability of natural plant pigments against environmental

factors could pose restriction to their utilization in industry. Therefore, an attempt was made in this study to assess the stability of the pigments under the influence of pH, temperature, metal ions ( $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$ ) and with supplementation of ascorbic acid, which may act as stabilizers.

### EXPERIMENTAL SECTION

#### Fruit samples

Fresh purple-red pitaya fruits (*H. polyrhizus*) used in this study was obtained from a fruit plantation in Johore, Malaysia. Upon arrival of fruits, they were washed and peeled to remove the skins. The fruit flesh was macerated in a Waring blender until completely homogenized and kept in plastic containers wrapped in aluminium foil and stored at  $-20$  °C prior to analysis. The peel parts were also stored under the same conditions as fruit flesh.

#### Solvents and reagents

Reagents and solvents of analytical or HPLC grade were obtained from Sigma-Aldrich, Merck or J.T. Baker.

#### Procedure

##### Pigment Extraction with several solvents

In this study, different solvents were used for pigment extraction in order to select the one that can

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give the highest extraction yield. The homogenized fruit flesh or peel was thawed prior to extraction. For pigment extraction from fruit flesh, solvents used were 80 % acetone, 80 % methanol and water. However, for peel component, only 80 % acetone and 80 % methanol were used for pigment extraction. During pigment extraction, 1 part of the sample (fruit flesh or peel) was shaken with 2 parts of solvent for a few minutes. It was allowed to stand for 15 min. Then, the seeds, raw fibres, mesocarp fibres and mucilaginous material were separated from the pigment extract by centrifugation at 3500 rpm for 15 min and filtration. The residue was re-extracted with the selected solvents in the same manner. The filtrate was combined with that obtained earlier and stored at -20 °C prior to betacyanin pigments quantification.

#### Quantitation of betacyanins

Red pitaya pigment content was measured in a similar way to that described by Cai *et al* [6], with slight modification. Quantification of betacyanins was carried out applying the equation  $Bc = [(A \times DF \times MW \times 1000 / \epsilon \times l)]$  where Bc is the betanin equivalents in mg/L, A is the absorption value at the absorption maximum ( $\lambda_{max} = 536$  nm), DF is the dilution factor, MW is the molecular weight of betanin (550 g/mol),  $\epsilon$  is the molar extinction coefficient of betanin (60,000 l/mol x cm), and l the pathlength of the cuvette. All determinations were performed in triplicate.

#### Pigment stability studies

Based on the yield of betacyanin content, water was selected as solvent for pigment extraction for the stability studies. The stability of betacyanin aqueous-based pitaya fruit extracts was evaluated under different pH values (2 to 10), temperature (25 to 75 °C), in presence of metal ions such as  $Cu^{2+}$  and  $Fe^{2+}$  (concentrations ranging from 0 to 150 ppm) and ascorbic acid (concentration 0 to 1.6 ppm). The pigment retention used to indicate colorant degradation as affected by these factors was calculated in each case after 12 hrs under the experimental conditions described above. Pigment retention (%) was calculated by the formula [betacyanin content after X storage time interval / betacyanin content at zero storage time] x 100. The effects of pH, metal ions and ascorbic acid on pigment retention were studied at 25 °C with sample inside capped vials covered with aluminium foil and sealed with parafilm. For the effects of temperature studies, test samples at pH 5.0 were immersed in a water bath and kept at various temperatures (25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75 °C) for 12 h. The test samples (10 mL) were prevented from the exposure of light and atmosphere as both factors could cause additional negative effects to the pigments. Triplicate measurements will be carried out.

#### Effects of supplementation with ascorbic acid

Betacyanin pigment stability improvement by supplementation of ascorbic acid at 0.23 % (w/w) concentration was evaluated at different temperatures (5 and 25 °C) over a storage period of 28 days. The pigment extracts were checked at several time intervals for pigment retention (%), color (lightness\*, a\*, b\*, chroma and hue angle<sup>o</sup>) and color retention. All analyses for colour stability and chromaticity were done in duplicate.

#### Colour analyses

Color was measured by colorimeter (CM 3500D) obtained from Minolta Co., Japan. Results were given on Commission Internationale de l'Eclairage L\*, a\*, and b\* (CIELAB) colour space coordinates. Hue angle [ $H^o = \tan^{-1} b^* / a^*$ ] and Chroma [ $C^* = (a^{*2} + b^{*2})^{1/2}$ ] were calculated from CIE a\* and b\* values using illuminant D<sub>65</sub> and 10° observer angle. All determinations were performed in duplicate. Color retention was also calculated according to the method of Cai & Corke [7] as follows:

[Chroma value at X storage time / Chroma value at zero storage time] x 100.

## RESULTS AND DISCUSSION

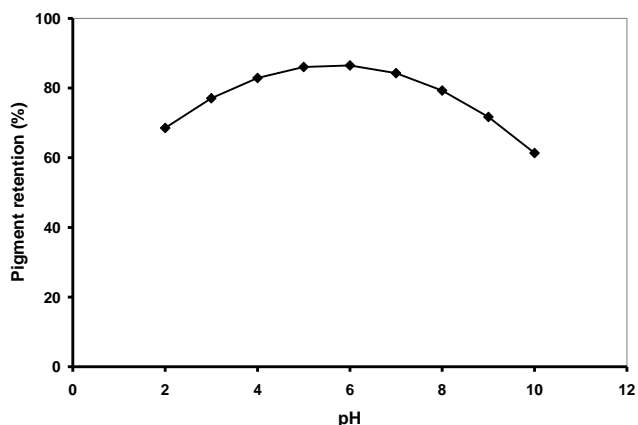
#### Total betacyanin content in pigment extracts

Total betacyanin pigments were calculated and tabulated in Table 1. For both peel and flesh components, the yield obtained using methanol 80 % as solvent was significantly ( $p < 0.05$ ) higher than with acetone. However, there was no significant differences of the yield obtained using methanol 80 % and water in extracting the desired pigments from fruit flesh. The concentration of betacyanins expressed as betanin equivalents per 100 g of flesh and peel were  $10.1 \pm 0.6$  mg and  $6.7 \pm 0.2$  mg, respectively when methanol 80% was used as solvent. The pigments contents in flesh agreed well with the values ( $10.3 \pm 0.22$  mg) reported by Wu *et al* for pitaya fruits (*H. polyrhizus*) cultivated in Taiwan [8], but were much lower than the values ( $28 \pm 1.9$  mg) reported by Wybraniec and Mizrahi for fruits grown in Israel [9].

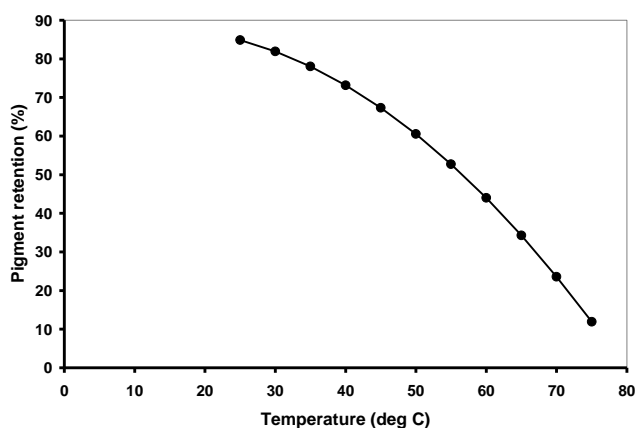
**Table 1.** Concentration of betacyanin pigments compounds using different solvents for extraction

Samples	Solvents	Betacyanin, (mg of BE/ 100g)
Fruit	80 % Acetone	$5.6 \pm 0.5^a$
Peels	80 % Methanol	$6.7 \pm 0.2^b$
Fruit	80 % Acetone	$7.5 \pm 0.4^c$
Flesh	80 % Methanol	$10.1 \pm 0.6^d$
	Water	$9.8 \pm 0.6^d$

Means with different letters are significantly different at  $p < 0.05$ . BE is Betanin Equivalents



**Fig 1.** Pigment retention (%) against pH at temperature 25 °C

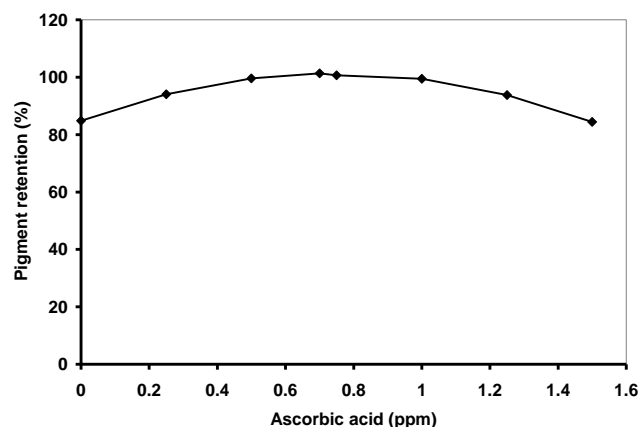


**Fig 2.** Pigment retention (%) against temperature at pH 5.0

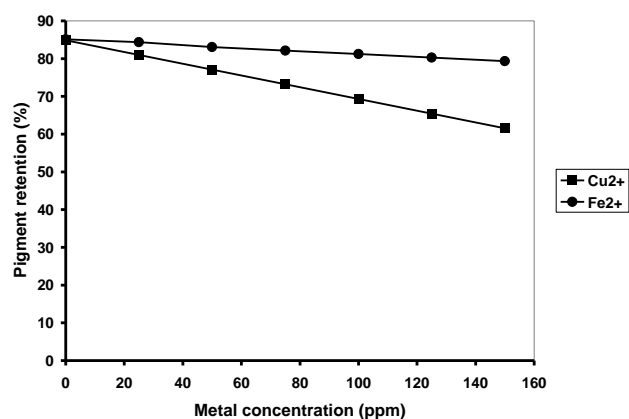
The fruits cultivated in Malaysia were more similar with that grown in Taiwan. However, the pigment contents in peel component was lower than the results ( $13.8 \pm 0.85$  mg) reported by Wu *et al* [8] who found that the peel exhibited higher pigments contents compared to flesh components.

#### Effects of pH, temperature, and metal ions on betacyanin pigment retention

For determination of betacyanin stability, overall % pigment retention was assessed. The results of pigment stability of fruit extracts from *H. polyrhizus* under the influence of different pH, temperature, ascorbic acid and metal ions ( $\text{Cu}^{2+}$  and  $\text{Fe}^{2+}$ ) are given in Fig 1, 2, 3 & 4, respectively. Analysis showed that betacyanin pigment was most stable at pH range between 5 and 6. Upon increasing the temperature from 25 to 75 °C pigment retention decreased with about 86% reduction at temperature of 75 °C. Maximum pigment stability was obtained for pitaya pigment preparations at pH 5.0 with ascorbic acid supplementation up to 0.7% affording a



**Fig 3.** Pigment retention against ascorbic acid at pH 5.0



**Fig 4.** Pigment retention (%) against metal concentration at pH 5.0 and 25 °C

pigment retention of about 99% after storing at 25 °C for 12 h (Fig 3). This suggested that supplementation with ascorbic acid could enhance the pigment stability against the detrimental effects caused by pH and temperature. Nevertheless, if the concentration of ascorbic acid exceeded 0.7 %, it may change its role from pigment stabilizer to become a pro-oxidant. The effects of metal ions (Fig 4) on betacyanin degradation was enhanced with  $\text{Cu}^{2+}$  exhibiting the greatest effect.

#### Betacyanin pigment stabilisation by addition of ascorbic acid

The effects of supplementation of ascorbic acid (0.25 %,) on betacyanin pigment stability and chromaticity values at pH 5.0 through time (for 28 days) were also investigated. The results are given in Fig 5 & 6 and Table 2. Addition of ascorbic acid to pigment preparations positively affected betacyanin stability. Higher storage stability at 5 °C (79.2% pigment retention) than at 25 °C without addition of ascorbic acid was observed after 28 days. However samples at 25 °C without ascorbic acid became cloudy after 2 days

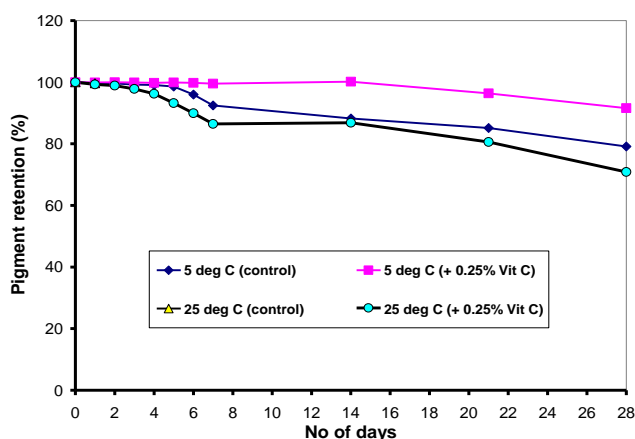


Fig 5. Pigment retention upon storage days

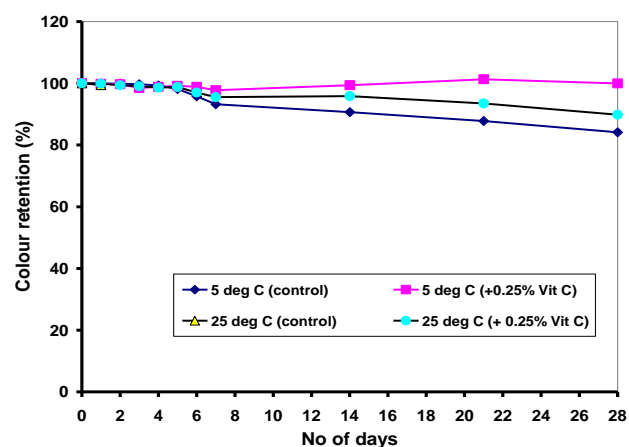


Fig 6. Colour retention upon storage days

Table 2. Colour properties of pitaya pigment extracts upon storage

Days	0	1	2	3	4	5	6	7	14	21	28
<b>Samples at 5 °C (control)</b>											
Lightness	52.26	52.09	52.18	52.05	52.37	52.70	53.16	54.12	56.24	57.12	58.05
a*	82.13	82.05	82.04	81.86	81.60	80.64	78.67	76.64	74.64	72.64	69.64
b*	-27.36	-27.43	-27.33	-27.24	-27.06	-26.80	-26.26	-25.26	-24.27	22.27	-21.27
Chroma*	86.57	86.52	86.47	86.28	85.97	84.97	82.94	80.69	78.49	75.98	72.82
Hue angle	341.57	341.52	341.57	341.59	341.66	341.62	341.54	341.76	341.99	342.96	343.01
<b>Samples at 5 °C (+ 0.25% ascorbic acid)</b>											
Lightness	52.26	52.09	51.87	51.57	51.67	52.13	52.32	51.90	51.43	52.31	52.69
a*	82.13	82.04	82.00	80.90	81.61	81.57	81.58	80.60	80.96	81.61	80.09
b*	-27.36	-27.10	-26.89	-26.83	-25.84	-26.82	-25.82	-25.72	-29.16	-32.03	-32.70
Chroma*	86.57	86.40	86.29	85.24	85.60	85.87	85.57	84.61	86.05	87.67	86.51
Hue angle	341.57	341.72	341.85	341.65	342.43	341.80	342.44	342.30	340.19	338.57	337.79
<b>Samples at 25 °C (control)</b>											
Lightness	52.26	52.56									
A*	82.13	81.69									
B*	-27.36	-27.10									
Chroma*	86.57	86.07									
Hue angle	341.57	341.65									
<b>Samples at 25 °C (+ 0.25% ascorbic acid)</b>											
Lightness	52.26	52.21	51.91	52.05	51.93	52.06	51.85	52.22	52.10	53.12	55.54
a*	82.13	81.76	81.12	80.51	79.56	78.96	76.99	75.12	74.97	72.85	69.81
b*	-27.36	-28.17	-28.66	-29.45	-30.89	-32.91	-33.51	-34.54	-35.54	-35.27	-34.28
Chroma*	86.57	86.47	86.03	85.73	85.34	85.54	83.97	82.68	82.97	80.94	77.77
Hue angle	341.57	340.99	340.54	339.91	338.78	337.37	336.48	335.31	334.64	334.16	333.84

thus measurements could not be made. Supplementation with ascorbic acid increased storage stability at 5 °C (91.6%) than at 25 °C (70.9%). Samples at 5 °C with addition of ascorbic acid did not show a decrease in pigment content for about 2 weeks compared to samples with ascorbic acid which showed a small reduction in pigment retention after about 5 days. This indicated that there is a stabilising effect of ascorbic acid on pigment retention. Fig 6 and Table 2 give results on the colour properties of pigment preparations. Greater colour retention at both temperatures in samples with ascorbic acid compared to samples without ascorbic

acid was found throughout (Fig 6). Addition of ascorbic acid was able to retard lightness increment in both samples at both temperatures as shown in Table 2. A decreasing C\* values was observed in samples at 25 °C with addition of ascorbic acid and also in samples without addition of ascorbic acid at 5 °C. Whereas samples at 5 °C with addition of ascorbic acid did not show any reduction in C\* values. A decreasing C\* value is indicative of yellow degradation products resulting from thermal treatment, thus causing a decline in colour purity.

## CONCLUSION

Red-purple pitaya extract was shown to display surprising pigment stability and nearly unaffected colour shade upon moderate thermal treatment. Since its pigments were previously shown to retain their appearance over a broad pH range (pH 3–7), application of purple pitaya as a natural food colorant appears to be promising.

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