Red-fleshed pitaya (*Hylocereus polyrhizus*) fruit colour and betacyanin content depend on maturity

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Abstract: Red-fleshed pitaya fruit is a potential fruit for betacyanins extraction. However, there is lack of report on profiles and total contents of betacyanins in the peel and flesh. The objectives of this study were to determine colour, total betacyanins content and its separation in the peel and flesh of red-fleshed pitaya fruit harvested at 25, 30 and 35 days after flower anthesis (DAA) and to examine the usefulness of tristimulus colour measurement as predictors of pigment content in red-fleshed pitaya fruits. There were significant relationships between DAA and colour (*L*, *C* and *h*° values), and total betacyanins contents of peel and flesh of red-fleshed pitaya fruit. A total of three types betacyanins were separated from peel and flesh of pitaya fruit at 30 and 35 DAA while for 25 DAA, only one type of betacyanins was separated. The total concentration of betacyanins in the fruit peel of 25, 30 and 35 DAA was 0.24, 3.99 and 8.72 mg/mL, respectively. The fruit flesh contains 2.40, 7.93 and 11.70 mg/mL betacyanins at 25, 30 and 35 DAA, respectively, which was higher than peel. The tristimulus measurements can be adequately used to estimate the total betacyanins content of peel and flesh of red-fleshed pitaya fruit instead of tedious pigment extraction methods.

Keywords: *L*°; *C*°; *h*°; betanin; days after flower anthesis

Introduction

The red-fleshed pitaya or dragon fruit (*Hylocereus polyrhizus*) is becoming popular in Malaysia due to its unique shape, attractive red colour and high functional properties. It is belongs to the family of Cactaceae and order of Caryophyllales. The peel and flesh of this species are red in colour. The flesh is delicate and juicy and contains numerous soft black seed. The red colour of pitaya fruit is attributed by betacyanins, which is a class of water-soluble pigments (Wybraniec *et al*., 2007). The red-violet betacyanins and the yellow betaxanthins belong to the betalain pigments, which are characteristics for plants of the order Caryophyllales. Betalains occur only in the plants from 10 families in the order Caryophyllales (Cai *et al*., 2005).

Due to strong consumer demand for more natural product which is more safety and health benefit because of the public concern about possible or proven harmful effects of artificial food colorants in food producing industries, thus the trend towards replacement of synthetic colorants by natural product has been increasing although it have higher cost (Boyd, 1998; Jackman and Smith, 1996). Nowadays, red beetroot (*Beta vulgaris*) are the main commercial source of betacyanins which available in the concentrated and powder form. However, red beetroot contains geosmin and pyrazines that are responsible for the unpleasant peatiness of this crop as well as high nitrate concentrations associated with the formation of carcinogenic nitrosamines, there is a demand for alternative compounds (Moshammer *et al*., 2005). In contrast to red beetroot, red-fleshed pitaya fruit does not have this negative sensorial impact. Alternatively, betacyanins from red-fleshed pitaya fruit may be a potential source on top of red beetroots.

In tomato, pigment synthesis is closely related to the initiation and progress of ripening, and the red colour is result from the accumulation of lycopene (Helyes and Pek, 2006). The CIELab system of *a*/*b*° was closely related with lycopene and can be used to characterize stages of maturity in fresh tomatoes. Pitaya fruit is a fast growing and developing fruit. Under Malaysia condition, the flesh of fruit turn from creamy white to full red-violet within 26-28 days after flower anthesis (DAA) while peel took 1-2 days longer for the green colour changed to red. The fruit is ready for harvest once the peel has turned full red but not later than 35 DAA as fruit start to crack and split and thus the quality deteriorate. The present work to
determine profiles and total contents of betacyanins used edible flesh of fruit that reached full ripening stage (Stintzing et al., 2002; Wybraniec and Mizrahi, 2002). In addition, there is little report on profiles and total contents of betacyanins in the peel and flesh as colour of fruit start turning into red. This information is useful in preparation for the emerging of peel and flesh pigment extraction industry. Therefore, the objectives of this study were to determine colour, total betacyanins content and its separation in the peel and flesh of red-fleshed pitaya fruit harvested at 25, 30 and 35 DAA and to examine the usefulness of tristimulus colour measurement as predictors of pigment content in red-fleshed pitaya fruits. This is because tristimulus colour measurements are easy to obtain, they often are used as descriptors for changes in pigment composition in lieu of extracting and measuring pigments themselves.

Materials and Methods

Plant Materials

Red-fleshed pitaya fruits (Hylocereus polyrhizus) were obtained locally from Yap Tee Pitaya Farm, Sepang, Selangor, Malaysia. Fruits were tagged immediately after hand-pollination. These fruits were harvested for analysis at 5 days interval beginning from 25 to 35 DAA. The harvested fruits were transported in air-conditioned car to Postharvest Laboratory, Universiti Putra Malaysia within 1 h and the experiment was carried out immediately once fruits reached laboratory.

Solvents and reagents

Solvents and reagents used were of laboratory and HPLC grades. Acetonitril, Folin-Ciocalteau reagent, trichloroacetic acid (TCA), sodium hydroxide and sodium potassium tartrate were purchased from MERCK (Darmstadt, Germany). Bovine albumin serum (BSA), Sephadex G-25, sodium carbonate and copper sulfate were obtained from SIGMA CHEMICAL Co. (St. Louis, MO, USA); meanwhile sodium dihydrogen orthophosphate anhydrous and sodium dihydrogen pyrophosphate anhydrous were from BDH Laboratory (Poole, England). Ethanol was obtained from Scharlau Chemie (Sentmenat, Spain). Betanin standard was purchased from ABCR (Karlsruhe, Germany).

Peel and flesh colour determination

Fruit peel and flesh colour of selected pitaya fruit were determined using Minolta CR-300 Chroma Meter (Minolta Corp., Osaka, Japan) using the Illuminare C (CIE, 1976) and results were expressed as lightness (L*), chroma (C*) and hue (h°). The L* value is ranging from 0 = black to 100 = white. The h° is an angle in a colour wheel of 360°, with 0°, 90°, 180° and 270° representing the hues red, yellow, green and blue, respectively, while C* is the intensity or purity of the hue. Zoned of 3 points on each fruit peel and flesh (3 cm x 3 cm) of this pitaya fruit representing a range of colour was labeled randomly with a felt pen, so that colour measurement was made on the same area of tissue that was used for pigment extraction. Total of 3 fruits with 9 zones of each harvesting day were used in each replication.

Pigment extraction

Each part of the fruit which had been zoned previously during colour determination was chopped into small pieces with a knife. Twenty gram of tissue from both peel and flesh component of each fruit was weighed separately using a weighing scale, then extracted respectively using 40 mL 80% aqueous ethanol in a speed blender for 5 min. The mixture was then filtered through a Whatman No. 1 filter paper, and the filtrate was collected.

The filtrate was then centrifuged (Beckman J2-21 centrifuge, SPINCO Inc., California, USA) at 12000 rpm under -4°C for 35 min. The extract was evaporated using a rotatory evaporator (Buchi Rotavapor R-200, BUCHI Laborttechnik AG., Flawil, Switzerland) at 25°C for another 35 min until 6 mL of initial volume remained. After concentrating, the extract of each sample was then kept in the 1 mL Eppendorf tube, wrapped with aluminum foil and stored at dark at -20°C. Then, the extract was used for betacyanins content determination and separation that was analyzed directly by UV-Vis spectrophotometer and high performance liquid chromatography (HPLC), respectively.

Total betacyanins content determination

UV-Vis absorption spectrum of betacyanins was determined by using a SECOMAM-PRIM Light-Vab-S/N 1143 spectrophotometer (Secomam CE., Domont Cedex, France) at wavelength of 538 nm. The betacyanins content was then calculated in a similar way to that reported by Wybraniec and Mizrahi (2002), with some modification by using the following formulas:

Betacyanins content (mg/100 g of fresh weight) = \( A_{538} \times \text{df} \times \text{v} \times 100 \times \text{w} / (\varepsilon \times \text{l}) \)

Where \( A_{538} \) = absorbance at 538 nm (\( \lambda_{\max} \)), L (path length) = 1.0 cm, DF = dilution factor, V = volume extract (mL), W = fresh weight of extracting material (g). For betanin, \( \varepsilon \) (mean molar absorptivity) = 6.5 x 10^4 L/mol cm in H_2O and MW= 550.
Red-fleshed pitaya (Hylocereus polyrhizus) fruit colour and betacyanin content depend on maturity

235

International Food Research Journal 16: 233-242

Extract separation

The concentrated betacyanins extract was separated prior to analytical using HPLC by gel filtration chromatography. The extract was separated by gel filtration on a Sephadex G-25 column (Escribano et al., 1998). The gel was allowed to swell in a 0.025 mol/L phosphate buffer (pH 5.7) for a day (Adams and von Elbe, 1977). The concentrated sample was then applied to a previously packed column (30 cm x 1 cm) of Sephadex G-25 that had been pre-equilibrated with phosphate buffer and material was eluted with the same buffer. One milliliter of red betacyanins fraction was collected and was then measured for its optical density at 538 nm. Only the fractions that had a highest value were stored and were subjected to HPLC analysis.

Betacyanins separation using HPLC method

Analytical HPLC was carried out by using an detector (JASCO UV-1570 Intelligent UV-Vis detector, IASCO Inc., MD, USA) which had been set at a wavelength of 538 nm equipped with LiChrospher 100RP-18 reversed phase column (5 μm, 250 mm x 4 mm i.d.) (MERCK KGaA., Darmstadt, Germany). For each analysis, 40 μL of the filtered sample was injected directly into the chromatographic column. Nine hundreds milliliters of 0.5% aqueous TFA with 100 mL of acetonitril was used as a mobile phase. The mixture was eluted for 20 min at a flow rate of 1.0 mL/min. All computations were performed using a JASCO-Borwin chromatograph data processing program. Quantification of betacyanins pigment content was carried out by calculating mean values obtained from 3 injections which were pooled using 3 fruits at each replication.

Statistical analysis

The experimental design was a completely randomize design with three replications of three fruit per replicate. Data was analyzed by using the analysis of variance. Differences within each factor were determined by least significant difference. Linear and quadratic regression were used to analyze the relationships between DAA and each variable, whereas correlation was used to analyze the relationship between each variable using Statistical Analysis System for Window v6.12.

Results and Discussion

The L* values of pitaya fruit peel decreased linearly with progressed of DAA (R² = 0.56) but the L* values of flesh increased then decreased as days progressed, resulting in a significant quadratic effect (R² = 0.97) (Figure 1a). Low values of L* indicated that pitaya fruit were darker in colour due to the development of reddish colour in both peel and flesh as pigmentation process occurred during maturation. Similar finding was reported in rose hips (Rosa dumalis and Rosa rubiginosa) (Uggla et al., 2005) and peach fruit (Prunus persia L.) (Lewallen and Marini, 2003) where the fruit darkened with decreasing of L* values as ripening day progressed. A drastic decreased of 45% in L* values of pitaya fruit flesh colour as DAA progressed from 25 to 30 (Figure 1a). This result indicated that there was a remarkable change in lightness of pitaya fruit flesh as DAA progressed. This change is in conjunction with the works of Mwithiga et al. (2007) who found that changes in the fruit flesh colour of tamarillo fruit were well pronounced with 44% of decrease in L* values as fruit ripeness progressed. Such change was most likely due to the interconversion of one kind of pigment into another. The increased in number and colour of seeds during fruit ripening may partially contributed to the darkening of pitaya fruit flesh as thousands of seeds were embedded within the red-violet flesh (Weiss et al., 1994).

<p>| Table 1. Correlation coefficients (r) for colour (L*, C* and h°), betacyanins content (BeC), protein content (PC) and betanin content (BC) of peel and flesh of red-fleshed pitaya fruit. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|</p>
<table>
<thead>
<tr>
<th>Peel</th>
<th>L*</th>
<th>C*</th>
<th>h°</th>
<th>BeC</th>
<th>BC</th>
<th>Flesh</th>
<th>L*</th>
<th>C*</th>
<th>h°</th>
<th>BeC</th>
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<td>C*</td>
<td>-0.75*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C*</td>
<td>-0.83*</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>C*</td>
<td>-0.83*</td>
<td>-</td>
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<tr>
<td>h°</td>
<td>0.67*</td>
<td>-0.90*</td>
<td>-</td>
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<td>h°</td>
<td>-0.97**</td>
<td>0.83*</td>
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<td>0.86*</td>
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<td>-0.65</td>
<td>0.86*</td>
<td>-</td>
<td>-0.99**</td>
<td>-</td>
<td>BeC</td>
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<td>0.78*</td>
<td>-0.84*</td>
<td>0.86*</td>
<td>-</td>
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<td>0.78*</td>
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<tr>
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<td>0.78*</td>
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<td>BC</td>
<td>0.76*</td>
<td>0.78*</td>
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<td>-</td>
<td>BC</td>
<td>0.76*</td>
<td>0.78*</td>
<td>-0.84*</td>
<td>0.86*</td>
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n = 9
L*=lightness, C* = chroma and ho = hue angle
*,** Significant or highly significant at P ≤ 0.05, respectively.

L*=lightness, C* = chroma and ho = hue angle
*,** Significant or highly significant at P ≤ 0.05, respectively.
Figure 1. The relationship between colour a) lightness (L*), b) chroma (C*) and c) hue angle (h°) of peel and flesh of red-fleshed pitaya harvested at 25, 30 and 35 days after flower anthesis. Each point represents mean of three measurements.
The C* values of both peel and flesh showed significant quadratic relationships as DAA progressed (Figure 1b). There were a drastic increased of 65.2 and 47.4% in C* values of pitaya fruit peel and flesh, respectively as DAA progressed from 25 to 30 (Figure 1b), indicating a more pronounced colour saturation developed in the peel and flesh of the fruit. The C* values reflect the saturation or vividness of colour where colour becomes more intense as evidence by the increasing of C* values (Lancaster et al., 1997). Similar finding was reported by Uggla et al. (2005) where C* values of rose hips increased significantly during early stage of harvesting but thereafter no significant increase was found.

The h° values of red-fleshed pitaya fruit peel showed significant quadratic relationship with DAA where a sharp decreased of 93% occurred as DAA progressed from 25 to 30 and thereafter no noticeable change was observed (Figure 1c). The decreasing values of h° in the pitaya fruit peel as DAA progressed reflected the development of reddish peel colour. According to Lewallen et al. (2003) as harvest date progressed, peel colour of the peach fruit changed from orange to orange–red as evidenced by the decreasing h° values. A similar finding also agreed with this result where h° values of the peel of ‘Kensington Pride’ mango was significantly decreased as fruit ripened from hard green stage to yellow stage of maturity (Lal et al., 2003).

The possible explanation for the colour change in fruit peel was associated with the enzymatic degradation of chlorophyll as fruit ripeness increased (Ding et al., 2007). This process involved the dissolution of thylakoids and stroma within the chloroplasts which caused the loss of structure of chloroplasts accompanied by degradation of chlorophyll. Thereby greatly reducing absorption of red and blue region in electromagnetic spectrum as chlorophyll absorbs light strongly in the red and blue regions (Taiz and Zeiger, 2006), hence, loss of green colour in fruit peel. On the other hand, the new synthesis pigment or the remaining pigment such as anthocyanin or carotenoid absorbs only the yellow, orange, red or blue and green region, respectively, leading to the development of peel colour as ripening process advanced. According to Nerd and Mizrahi (1997) the changes of peel colour to pale green then to reddish of pitaya fruit was due to the changes in peel chlorophyll content during fruit developing. The concentration of chlorophyll a was 2.5 times higher than that of chlorophyll b in the younger pitaya fruit and both chlorophylls decreased markedly toward the end of fruit growth, followed by new colour pigmentation. For red-fleshed pitaya fruit which has a red scale, the increase in scale pigment parallel to the development of peel colour (Nerd et al., 1999). In addition, the first change in peel colour was at 24-25 DAA in Hylocereus undatus and 26-27 days in Hylocereus polyrhizus, and then the peel turn fully red 4-5 days after the first colour change in both species. All these explanations can possibly describe the colour changes in peel of red-fleshed pitaya fruit from light green to reddish as h° values of peel colour decreased parallel to the progressed of DAA from 25 to 35.

There was also significant quadratic relationship between DAA and h° values of pitaya flesh colour (R² = 0.81) (Fig. 1c). As DAA progressed, the h° values of flesh colour increased gradually suggesting the development of red-violet colour in fruit flesh and colour shifted from reddish purple to purplish red. The formation of pigment which caused colour changes in the peel as well as flesh of the fruit is one of the important changes as the fruit goes through the last stage of development or maturation. As reported by Whale et al. (2008) the colour changes in ‘Cripp’s Pink’ apple was due to degradation of chlorophyll and an increase in the synthesis of total anthocyanin and cyanidin 3-galactoside. According to Wybraniec et al. (2001) the most important colour pigment found in most cacti fruits are betacyanins which caused the fruit flesh colour changes as DAA progressed. Consequently, all these findings can possibly describe the h° values of peel and flesh of pitaya fruit as colour changes from green to reddish as ripening progressed.

Total betacyanins content

Total betacyanins content of both peel and flesh of red-fleshed pitaya fruits showed significant quadratic relationships with DAA (R² = 0.992 and 0.994, respectively) (Figure 2). There were drastic increased of 90 and 65.13% of total betacyanins content in peel and flesh, respectively as DAA progressed from 25 to 30, thereafter the increase was slow. This result indicated that total betacyanins content increased as DAA progressed i.e. betacyanins being synthesized. Synthesis of betacyanins giving a red-violet colour to pitaya fruit (Strack et al., 2003) as it is a pigment that cause a red-violet colour in fruits, flowers and vegetative organs of the plants (Stintzing and Carle, 2004; Wybraniec and Mizrahi, 2002). Nerd and Mizrahi (1997) also reported that in many cultivars of cactus fruits, the peel colour is similar to those of the flesh, but pigmentation starts earlier in the flesh. This finding can possibly describe the increased of total betacyanins content in pitaya fruit flesh which was more pronounced than peel as DAA progressed from 25 to 30, due to earlier synthesis of betacyanin pigment in fruit flesh which caused the pitaya fruit flesh turned reddish prior in the peel.
Table 2. Retention time, area of peak, concentration and total concentration of betacyanins for peel and flesh of red-fleshed pitaya fruit at different day after anthesis.

<table>
<thead>
<tr>
<th>Day after anthesis</th>
<th>Pigment</th>
<th>Peel</th>
<th></th>
<th></th>
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<tr>
<td></td>
<td></td>
<td>Retention time (min)</td>
<td>Peak area (µV*s)</td>
<td>Concentration (mg/mL)</td>
<td>Total concentration (mg/mL)</td>
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<td>Concentration (mg/mL)</td>
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<tr>
<td>25</td>
<td>Betanin</td>
<td>9.6</td>
<td>11866.8</td>
<td>0.244</td>
<td>0.244 e</td>
<td>9.683</td>
<td>116398</td>
<td>2.397</td>
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<tr>
<td></td>
<td>Isobetanin</td>
<td>-</td>
<td>-</td>
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<td>-</td>
<td>-</td>
<td>-</td>
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<tr>
<td>30</td>
<td>Unknown</td>
<td>5.7</td>
<td>58476.8</td>
<td>-</td>
<td>0.244 e</td>
<td>5.767</td>
<td>175436</td>
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<td>123605</td>
<td>2.545</td>
<td>5.52</td>
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<td>70191.5</td>
<td>1.445</td>
<td>3.99 b</td>
<td>13.258</td>
<td>117234</td>
<td>2.414</td>
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<td>35</td>
<td>Unknown</td>
<td>5.8</td>
<td>70045.3</td>
<td>-</td>
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<td></td>
<td>13.423</td>
<td>177063</td>
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</tbody>
</table>

^Total concentration was the sum of concentration of betanin and isobetanin which obtained from betanin standard curve.
^Mean followed by the same letter within each column are not statistically different by LSD at P ≤ 0.05.
**Highly significant at P ≤ 0.05.
Red-fleshed pitaya (Hylocereus polyrhizus) fruit colour and betacyanin content depend on maturity

There was significant negative correlation between total betacyanins content and L* values of pitaya fruit flesh (Table 1), indicating as DAA progressed; fruit were darker in colour with increased of total betacyanins content. This result is in agreement with Mößhammer et al. (2005) who reported that L* values constantly declined with increased of betacyanins ratios. A similar observation has also been made by Lancaster et al. (1997) when a significant linear relationship was detected between log (chlorophyll) and L* values, indicating a logarithmic relationship between increasing chlorophyll and darkness of the material. Besides that, total betacyanins content of pitaya fruit was positively significant correlated with C* values of fruit peel and flesh (Table 1). The possible explanation of this correlation is that the fruit became more intense and vivid in colour due to synthesis and accumulation of the betacyanins pigment in the cell vacuoles during ripening.

There was a negative significant correlation between total betacyanins content and h° values of pitaya fruit peel but contrast finding was found in flesh (Table 1). This showed that h° values of peel colour declined with increase of total betacyanins content. The possible explanation of this correlation is that peel constantly loss its green colour which due to the loss of chlorophyll content and followed by synthesis of betacyanins. On the other hand, when h° values of flesh colour increased, fruit flesh turned to reddish purple with increased of total betacyanins content as DAA progressed. The formation and development of peel and flesh reddish colour could be due to betacyanins structure that consist of cyclo-DOPA aromatic structure and it links to the betalamic acid which responsible for the deep red-violet colour of this pigment (Wybraniec et al., 2007). Thus, peel and flesh turned reddish in colour with increase of total betacyanin content. This result agrees with the works of Nerd et al. (1999) who found that the development of flesh colour was accompanied by an increase in the content of water-soluble pigment in the red-fleshed pitaya fruit. A similar relationship also proposed by Lancaster et al. (1997) who investigated the relationship between pigments and colour scales of various fruits and vegetables, found that there was a linear relationship between anthocyanin concentration and h° values in anthocyanin-contained tissues.

**Betacyanins separation**

For fruit harvested at 25 DAA, only single peak can be observed at 9.6 min for both peel and flesh of the fruit (Table 2). While fruit harvested at 30 and 35 DAA, three major peaks can be observed by eluting at about 5.7 (peak 1), 9.6 (peak 2) and 13.2 min (peak 3) for both peel and flesh of pitaya fruit. The smaller peaks in both chromatograms, eluting before peak 1 are believed to be peaks of mobile phase. Schliemann et al. (1996) reported in their works on betacyanins of Phytoleacca americana (pokeberry) that the ratio of prebetanin (betanin 6-O-sulphate) to betanin depend on the ripening stage of the fruits. Furthermore, according to Fernandez-Lopez and Almela (2001), the absence of one of the peaks of betacyanins in Opuntia ficus-indica (prickly pear fruit) at 533 nm
would indicate that betacyanins are to be found in these fruits in a very low level. The findings by Schliemann et al. (1996) and Fernandez-Lopez and Almela (2001) can possibly explain the elution of single peak that appear in pitaya fruit at 25 DAA. From the respective retention time in comparison to commercially obtained betanin standard, peak 2 was identified as betanin while peak 3 was isobetanin whereas peak 1 was unknown due to unavailable of other standard (Table 2). Betanin (betaninidin-O-β-glucoside) is the best known betacyanins (Gandia-Herrero et al., 2005), first described in red beetroot (Wyler and Dreiding, 1957). Betanin was eluted first, followed by isobetanin (isobetaninidin-O-β-glucoside) (Herbach et al., 2006). This was due to hydrophobic effect as betanin was a more polar betacyanins glycons, so was eluted first, meanwhile isobetanin was less polar aglycons (Wybraniec et al., 2007), which the latter created a hydrophobic interaction with the profile agreed well with mobile phase and stationary phase that caused the molecule spends some time moving down the column in the mobile phase. The reversed-phase HPLC eluted that predicted by reversed-phase chromatography principle where branched chain compounds elute more rapidly than their corresponding isomers because the overall surface area is decreased (Kujala et al., 2002). The configuration of C-15 epimers, isobetanin (Cai et al., 1998) (peak 3) allows greater interaction with the stationary phase and therefore has a greater interaction retention value. This result is similar to early results reported by Schwartz and von Elbe (1980) whom identified the order of elution of betacyanins from red beet as betanin, isobetanin, betanidin and isobetanidin, respectively. Cai et al. (1998) also reported the order of elution of betacyanins from Amaranthus species as amaranthine, isoamaranthine, betanidin and isobetanidin, respectively. Hylocereus costaricensis possessed the order of elution of betacyanins as betanin, isobetanin, phyllocactin, isophyllocactin, hylocerenin and isohylocerenin, respectively as detected by reversed-phase HPLC (Wybraniec and Mizrahi, 2002). Wybraniec and Mizrahi (2002) and Wu et al. (2006) obtained at least 6 and 4 peaks, respectively in profiling Hylocereus polyrhizus pigment pattern, which in this study only 1 to 3 peaks were obtained. This was due to extraction conditions and purification steps prior to analysis (Berg et al., 2002) as well as different type of column and detector being used (Wybraniec and Mizrahi, 2002).

There were significant differences in total betanin concentration of fruit peel and flesh as DAA progressed (Table 2). Both peel and flesh of pitaya fruits at 35 DAA had a significant higher total betanin concentration than those at 25 and 30 DAA, respectively (Table 2). There were positive correlation between betacyanins content and betanin concentration in peel and flesh of the fruit (Table 1). This result indicated that the content of betacyanins and betanin increased simultaneously as DAA progressed.

**Conclusion**

In short, as DAA progressed from 25 to 35 DAA, the peel colour of pitaya fruit turned from green to red, while the flesh turned from creamy white mixture with red to red-violet with tremendous changes found in fruit harvested at 25 and 30 DAA. The peel and flesh colour changes coincided with increase of protein content, total betacyanins content and types of betacyanins being separated. The tristimulus measurements can be adequately used to estimate the total betacyanins content of peel and flesh of red-fleshed pitaya fruit instead of tedious pigment extraction methods. The peel of red-fleshed pitaya fruit also contains betacyanin which can be developed into beauty and health products and food natural colourants as its flesh and hence reduce the food wastage.

**Acknowledgements**

This research was sponsored by Malaysia Toray Science Foundation.

**References**


Red-fleshed pitaya (Hylocereus polyrhizus) fruit colour and betacyanin content depend on maturity


