

## Bioactive Compounds and Total Antioxidant Capacity of Different Tissues of Two Pitaya (Dragon Fruit) Species Grown in Sri Lanka

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### ABSTRACT

Bioactive compounds (*viz.* total phenolics, total flavonoids and vitamin C) and total antioxidant capacity (TAC) of flesh and peel of two dragon fruit species namely, *Hylocereus polyrhizus* (red-fleshed) and *Hylocereus undatus* (white-fleshed) were investigated. TAC was determined by Ferric reducing antioxidant power (FRAP) assay. Colorimetric methods were used to determine total phenolic (Folin-ciocalteu method) and total flavonoid contents whereas vitamin C content was measured using a titration method (titration with 2,6-dichloro-phenolindophenol dye). Several selected physical and chemical properties of the two dragon fruit species were also determined. Flesh of red-fleshed dragon fruit had significantly higher amounts of total phenolics ( $51.80 \pm 1.64$  mg GAE/100 g FW) and total flavonoids ( $46.29 \pm 2.47$  mg RE/100 g FW) and higher TAC ( $67.25 \pm 3.20$  mg TE /100 g FW), whereas higher vitamin C content ( $31.11 \pm 3.85$  mg / 100 g FW) was observed in flesh of white-fleshed dragon fruit. Flesh of two dragon fruit species showed significantly higher bioactive compounds and TAC than their peels. Significantly higher linear relationships were found between the TAC values and the total phenolic content ( $R^2 = 0.907$ ,  $p < 0.001$ ) and total flavonoid content ( $R^2 = 0.888$ ,  $p < 0.001$ ). However TAC poorly correlated with vitamin C content. Findings of the current study indicate that flesh of both dragon fruit species contained higher amounts of total phenolic, total flavonoid, vitamin C contents

and higher TAC than peel. Significantly, higher total phenolic, total flavonoid contents and TAC were observed in flesh of red-fleshed dragon fruit than in white-fleshed dragon fruits. Flesh of white-fleshed dragon fruit had high vitamin C content, compared to red-fleshed dragon fruits.

**Keywords:** Antioxidant Capacity, Dragon Fruit, Flavonoids, Phenolics, Vitamin C

### INTRODUCTION

Fruit of *Hylocereus* species known as Pitaya or Pitahaya belongs to the genus *Hylocereus* the botanical family *Cactaceae* which is native to Mexico, Central and South America (Bellec *et al.*, 2006). The few species of the genus *Hylocereus*, which consists of climbing three-ribbed stems and mostly white, fragrant, night blooming flowers, have been recently developed as fruit crops (Mizrahi *et al.*, 1997). In Asia, Pitaya is often called “Dragon fruit” following its bright red skin with green overlapping fins covering the fruit.

Each species of Dragon fruit may be distinguished from another by either the colour of the pulpy skin (exocarp), and /or the colour of the soft edible flesh (mesocarp and endocarp) which contains the seeds. Three species that have been commercialized are *Hylocereus undatus*, which has red-skinned fruit with white-flesh, *Hylocereus polyrhizus*, which has

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red-skinned fruit with red-flesh, and *Hylocereus megalanthus*, which has yellow-skinned fruit with white-flesh (Barbeau, 1990).

Dragon fruit has been reported as a source of bioactive compounds such as polyphenols, vitamin C, and antioxidant pigments etc. (Wu *et al.*, 2006; Choo and Yong, 2011). In red-fleshed Dragon fruit, the most important antioxidant pigments are the betacyanins and betaxanthins (Wybraniec *et al.*, 2001). Dragon fruit, as in many other fruits and vegetables, is also rich in antioxidants that help to reduce the incidence of degenerative diseases such as arthritis, arteriosclerosis, cancer, heart diseases, inflammation and brain dysfunction (Vaiserman, 2008).

Peeled fruit slices of Dragon fruit are used as fresh fruits desserts after meals in the hotels. Also, it can be used to produce jam, ice cream, jelly, fruit juice as well as wine (Anon, 2011). The pulp of red-fleshed Dragon fruit is already used in Israel for the production of red-violet colored ice cream and in low temperature dairy drinks (Wybraniec *et al.*, 2001).

Red-fleshed Dragon fruits have recently drawn much attention of growers worldwide, not only because of their red-purple colour and economic value as food products, but also because of their antioxidant activity (Wybraniec and Mizrahi, 2002). Since there is developing market in Europe as well as in Asia (Singapore, Hong Kong, Taiwan, Philippines, Malaysia and Thailand),

recently Dragon fruit has been widely cultivated in Nicaragua, Colombia, Israel, Australia, USA (Wu *et al.*, 2006) and Asia, including Taiwan Vietnam, Malaysia and South China (Mizrahi *et al.*, 1997).

Sri Lanka also began to grow this species in 1990s as a small scale crop and it is evident that the potential for expansion is very high in the wet and intermediate zones (*viz.* Bulathsinhala, Gampaha and Makandura). It could be grown in the intermediate and dry zones if irrigation is provided during the dry period (Gunasena and Pushpakumara, 2006).

However, there is no information available on amounts of bioactive compounds and antioxidant capacities of different tissues of dragon fruits species which are cultivated under Sri Lankan conditions. Therefore, this study was carried out to determine the bioactive compounds and antioxidant capacities of flesh and peel of red-fleshed and white-fleshed dragon fruit species grown in Sri Lanka.

## **MATERIALS AND METHODS**

### ***Materials***

The well ripen dragon fruits of red-fleshed (*Hylocereus polyrhizus*) and white-fleshed (*Hylocereus undatus*) were harvested on April 27, 2011 and on May 30, 2011 from farmers' field in Minuwangoda and Athurugiriya respectively. They were directly transported to the laboratory. The experiment was carried out in the laboratory of the Department of Plantation

Management, Wayamba University of Sri Lanka, Makandura, Gonawila (NWP) from March to July 2011.

### ***Sample Preparation***

The fruits were peeled manually. Separated peel and flesh of fruit samples were frozen in liquid nitrogen and then stored at -20 °C prior to analysis. A Completely Randomized Design (CRD) with three replicates was used in the experiment.

### ***Determination of Physical and Chemical Properties***

Fruit dimensions (length and diameter), fresh weight of peel and flesh of two dragon fruit species (n=10) were recorded separately. Moisture content of peel and flesh were determined by drying at  $80 \pm 2^\circ\text{C}$  until they reached a constant weight. Total Soluble Solid (TSS) content and pH value in flesh were determined using hand refractometer (Atago N-1E, Japan) and digital pH meter (Cyberscan 500, Singapore) respectively.

### ***Extraction Procedure***

All of the dragon fruit samples were extracted and analyzed in triplicate. Phenolic compounds were extracted by modifying a previously published method (Sandamali and Abeysinghe, 2010) as described below. Frozen ground dragon fruit samples (1 g) were weighed into 15 ml Teflon centrifuge tubes and 5 ml of chilled 80% methanol was added. The samples were then vortexed for 2 min and

centrifuged at 5,000 rpm for 10 min. Extraction procedure was repeated once and both supernatants were pooled and stored at -20°C until the determination of total phenolics, total flavonoids and total antioxidant capacity.

Vitamin C was extracted according to a procedure reported by Sandamali and Abeysinghe (2010). Briefly, vitamin C in fruit flesh and peel of dragon fruit were extracted from 1 g of ground samples using 11 ml of 1% (w/v) oxalic acid and the mixture was vortexed for 2 min. The solution was then centrifuged at 5,000 rpm for 10 min. The supernatant was stored at 4 °C until analysis was carried out.

### ***Determination of Total Phenolic Content***

The total phenolic contents of flesh and peel samples of red- and white-fleshed dragon fruits were determined using a modified Folin-Ciocalteu method (Abeysinghe *et al.*, 2007). Briefly, 0.5 ml of appropriately diluted samples or a standard solution of gallic acid was added to a 15 ml centrifuge tubes containing 4 ml of distilled water. Folin-Ciocalteu phenol reagent (0.5 ml) was added to the mixture and mixed by shaking. After 3 min, 1 ml of saturated sodium carbonate was added with mixing and incubated for 2 hr at 30 °C. The absorbance relative to that of a prepared blank was read at 760 nm using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). The total phenolic contents was expressed in mg of gallic acid equivalents (GAE) per 100 g fresh weight (FW) as standard.

### ***Determination of Total Flavonoid Content***

The flavonoid contents of the dragon fruit samples were measured by a colorimetric method (Wolfe and Liu, 2003) with modifications. A volume of 0.5 ml of a known dilution of extract was added to a test tube containing 3.5 ml of distilled water and mixed with 0.3 ml of 5 % NaNO<sub>2</sub>. After 6 min, 0.3 ml of 10 % Al(NO<sub>3</sub>)<sub>3</sub> solution was added; the mixture was allowed to stand for another 6 min, and then 2 ml of 2 M NaOH was added. The reaction mixture was diluted with another 1.4 ml distilled water and the absorbance of the mixture at 510 nm was measured immediately using a spectrophotometer (Shimadzu, UV Mini 1240, Japan). Total flavonoid content was calculated using the standard rutin curve and expressed as milligrams of rutin equivalents (RE) per 100 g of fresh weight (FW).

### ***Determination of Vitamin C Content***

Vitamin C contents of peel and flesh of dragon fruit extracts were determined by a titrimetric method (Sandamali and Abeysinghe, 2010). To summarize, 10 ml of dragon fruit extract was placed in a conical flask and titrated with freshly prepared 2,6-dichlorophenolindophenole (DCP) dye solution (0.5 g/100 ml) until a light but distinct rose pink colour appears and persists for more than 5 seconds. The volumes of used DCP dye were compared to those of vitamin C standard solution (500 ppm). The vitamin C content was expressed as mg/100g fresh weight of fruit peel or flesh.

### ***Determination of Total Antioxidant Capacity (TAC)***

TAC was determined using the Ferric Reducing Ability of Plasma (FRAP) assay (Benzie and Strain, 1996) with slight modifications. Briefly, the FRAP reagent was freshly prepared by mixing 25 ml of 300 mM Sodium Acetate buffer (pH 3.6), 2.5 ml of 10 mM 2,4,6-Tris(2-pyridyl)-s-triazine (TPTZ) solution, and 2.5 ml of 20 mM Ferric Chloride solution. The absorbance at 593 nm was read 4 min after mixing of 100 µl of fruit peel or flesh extract with 900 µl of FRAP reagent, using spectrophotometer (Shimadzu, UV Mini 1240, Japan). The measurement was compared to a standard curve of prepared trolox solutions and expressed as mg trolox equivalents (TE)/100g fresh weight (FW) of fruit peel or flesh.

### ***Statistical Analysis***

Data were analyzed according to a completely randomized design (CRD), with three replicates. Data were subjected to analysis of variance (ANOVA) using the general linear models (GLM) procedure of SAS, followed by Duncan's multiple-range test ( $p < 0.05$ ) (SAS Institute, 1999). Correlation coefficients were determined to describe the relationship between contents of bioactive compounds and TAC.

## **RESULTS AND DISCUSSION**

### ***Physical and Chemical Properties***

Red-fleshed dragon fruit showed a higher flesh weight ( $255.8 \pm 61.9$  g) than the white-

fleshed dragon fruit ( $205.1 \pm 60.4$  g) whereas, a higher peel weight ( $126.7 \pm 23.9$  g) was observed in white-fleshed dragon fruit than in red-fleshed dragon fruit ( $61.2 \pm 18.3$  g) (Table 1). White-fleshed dragon fruit peel and flesh contained a higher water content ( $91.7\% \pm 0.3$  and  $81.7\% \pm 1.0$ ) than peel ( $88.5\% \pm 1.2$ ) and flesh ( $80.4\% \pm 2.0$ ) of red-fleshed dragon fruit. White-fleshed dragon fruit contained higher TSS ( $13.3 \pm 1.3$ ) than red-fleshed dragon fruit ( $12 \pm 1.0$ ) whereas, pH value of red-fleshed dragon fruit ( $5.6 \pm 0.1$ ) was higher than that of white-fleshed dragon fruit ( $4.1 \pm 0.1$ ).

### ***Bioactive Compounds***

The two species of dragon fruits showed significantly higher total phenolic contents in flesh than in peel (Table 2). Flesh of red-fleshed dragon fruit showed significantly higher total phenolic contents ( $51.80 \pm 1.64$  mg GAE/100g FW) than the flesh of white-fleshed dragon fruit ( $32.37 \pm 1.72$  mg GAE / 100 g FW).

The higher phenolic content in red-fleshed dragon fruit was probably due to betacyanins, the pigments with phenolic structures found in red-fleshed dragon fruit (Wybraniec *et al.*, 2001). Our results of total phenolic content in red-fleshed dragon fruit were comparable with results of Wu *et al.* (2006). The total phenolic content of peel of red-fleshed dragon fruit ( $32.40 \pm 1.65$  mg GAE / 100 g FW) was significantly higher than that of white-fleshed dragon fruit ( $29.10 \pm 1.30$  mg GAE / 100 g FW).

Flesh of two dragon fruit species had

higher flavonoid contents than the peel of those species (Table 2). The total flavonoid content of flesh of red-fleshed dragon fruit ( $46.29 \pm 2.47$  mg RE/100 g FW) was significantly higher than that of white-fleshed dragon fruit ( $26.71 \pm 4.46$  mg RE/100 g FW). There were no significant differences in the flavonoid contents of the peels of two species. However, the peels of red-fleshed dragon fruit showed a higher flavonoid content ( $28.38 \pm 14.18$  mg RE/100 g FW) than the peel of white-fleshed dragon fruit ( $22.90 \pm 1.49$  mg RE/100 g FW).

There were no significant differences in the vitamin C content of peel or flesh of two dragon fruit species (Table 2). However, two species of dragon fruits showed higher vitamin C contents in flesh than in peel. Vitamin C contents of flesh of white-fleshed dragon fruit ( $31.11 \pm 3.85$  mg / 100g FW) were higher than that of red-fleshed dragon fruit ( $20.00 \pm 1.33$  mg / 100g FW). These findings on vitamin C content in white-fleshed dragon fruit was consistent with results of vitamin C content in dragon fruit flesh reported by Choo and Yong (2011).

### ***Total Antioxidant Capacity (TAC)***

The TAC for the flesh samples of the two dragon fruit species was considerably higher than that of peel samples in agreement with the total phenolics (Table 2). The TAC of flesh of red-fleshed dragon fruit ( $67.25 \pm 3.20$  mg TE /100g FW) was significantly higher than the flesh of white-fleshed dragon fruit ( $39.63 \pm 2.61$  mg TE

**Table 1.** Some physical and chemical properties of red- and white-fleshed Dragon fruit

<b>Characteristic</b>	<b>Red-fleshed dragon fruit</b>	<b>White-fleshed dragon fruit</b>
Fruit length (cm)	10.0 ± 0.9 <sup>a</sup>	11.7 ± 1.0 <sup>b</sup>
Fruit diameter (cm)	7.7 ± 0.6 <sup>a</sup>	7.5 ± 0.8 <sup>a</sup>
Flesh weight (g)	255.8 ± 61.9 <sup>a</sup>	205.1 ± 60.4 <sup>a</sup>
Peel weight (g)	61.2 ± 18.3 <sup>a</sup>	126.7 ± 23.9 <sup>b</sup>
Moisture in flesh (%)	80.4 ± 2.0 <sup>a</sup>	81.7 ± 1.0 <sup>a</sup>
Moisture in peel (%)	88.5 ± 1.2 <sup>a</sup>	91.7 ± 0.3 <sup>b</sup>
Total soluble solids (TSS) (%)	12 ± 1.0 <sup>a</sup>	13.3 ± 1.3 <sup>b</sup>
pH	5.6 ± 0.1 <sup>a</sup>	4.1 ± 0.1 <sup>b</sup>

Means with different letters in the row represent significant differences at  $P < 0.05$

**Table 2.** Contents of total phenolics, total flavonoids, vitamin C and total antioxidant capacity in flesh and peel of two dragon fruit species

	<b>Total Phenolics</b>	<b>Total Flavonoids</b>	<b>Vitamin C</b>	<b>TAC</b>
Flesh (red-fleshed)	51.80 ± 1.64 <sup>a</sup>	46.29 ± 2.47 <sup>a</sup>	20.00 ± 1.33 <sup>a</sup>	67.25 ± 3.20 <sup>a</sup>
Peel (red-fleshed)	32.40 ± 1.65 <sup>b</sup>	28.38 ± 14.18 <sup>b</sup>	17.78 ± 7.70 <sup>a</sup>	42.41 ± 10.52 <sup>b</sup>
Flesh (white-fleshed)	32.37 ± 1.72 <sup>b</sup>	26.71 ± 4.46 <sup>b</sup>	31.11 ± 3.85 <sup>a</sup>	39.63 ± 2.61 <sup>b</sup>
Peel (white-fleshed)	29.10 ± 1.30 <sup>c</sup>	22.90 ± 1.49 <sup>b</sup>	18.33 ± 6.11 <sup>a</sup>	32.66 ± 0.09 <sup>b</sup>

Means with different letters in the Column represent significant differences at  $P < 0.05$ .

/100g FW). There was no significant difference in TAC of peel of the two dragon fruit species. However, white-fleshed dragon fruit peel showed lowest TAC ( $32.66 \pm 0.09$  mg TE /100g FW).

In flesh and peel of both species, TAC showed highly significant correlations with the total phenolics ( $R^2 = 0.907$ ,  $p < 0.001$ ) and total flavonoid ( $R^2 = 0.888$   $p < 0.001$ ). These strong correlations suggested that phenolic components represent a significant contribution to the dragon fruit antioxidant activity. Similar strong relationship between phenolic content and TAC of red-fleshed dragon fruit was observed by Wu *et al.* (2006). However, a poor correlation between TAC values and vitamin C contents of dragon fruit ( $R^2 = 0.063$   $p < 0.67$ ) indicating that dragon fruit's vitamin C contents make a very low contribution to the fruit TAC as compared to the total phenolic content.

## CONCLUSION

Flesh of both dragon fruit species contained higher amounts of total phenolics, total flavonoids, vitamin C and a higher total antioxidant capacity than peel. Significantly, higher total phenolics, total flavonoids and total antioxidant capacity were observed in flesh of red-fleshed dragon fruit than white-fleshed dragon fruit. Flesh of white-fleshed dragon fruit had high vitamin C content when compared with red-fleshed dragon fruit.

Total antioxidant capacity significantly correlated with the total phenolic contents

and total flavonoid contents whereas, total antioxidant capacity poorly correlated with vitamin C contents which showed significantly higher contribution of phenolic compounds to the TAC of dragon fruits.

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