

## Variability in nutritional composition and phytochemical properties of red pitaya (*Hylocereus polyrhizus*) from Malaysia and Australia

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### Abstract

The present work sought to investigate the nutritional composition and phytochemical properties of red pitaya (*Hylocereus polyrhizus*) juices from Malaysia and Australia and to determine the optimum ethanol concentration (in the range of 0 – 100% ethanol) for the extraction of phenolic, flavonoid and betacyanin contents. The predominant macronutrient in red pitaya juice was carbohydrate while potassium and vitamin A were the major mineral and vitamin content. Red pitaya juice from Malaysia achieved optimal total phenolic content at 20% of ethanol (20 mL ethanol in 100 mL water, v/v); total flavonoid content at 60% (v/v); and betacyanin content at 0% (v/v). Red pitaya juice from Australia achieved the maximum total phenolic content at 60% (v/v); total flavonoid content at 20% (v/v); and betacyanin content at 80% (v/v). Nutritional composition and the phytochemical properties of red pitaya in Malaysia and Australia were significantly different suggested the role of environmental factors like soil and climate on the phytochemical properties of red pitaya.

### Keywords

*Hylocereus polyrhizus*  
Nutritional composition  
Phenolic content  
Flavonoid content  
Betacyanin contents

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### Introduction

Fruits and vegetables contain numerous natural antioxidants that offer protection against chronic diseases. Other than vitamins, fibre, and minerals, polyphenolic compounds were found to be the most abundance antioxidants in plants particularly in the tropical fruits (Rufino *et al.*, 2010). Evidences suggested that the phytochemical and antioxidant properties of plants vary in different geographical regions (Connor *et al.*, 2002; Antonen *et al.*, 2005; Nurul and Asmah, 2012). Besides, factors such as cultivar, season, climate, agriculture practices, water availability, transport, handling and storage of the plants may affect the bioactive compounds (Franke *et al.*, 2004; Wall, 2006). This variability explains the need of food composition data at the national level. Moreover, the chemical constituents of foods is the first key elements to be consider in the dietary treatment and prevention of diseases as well as in any study of human nutrition. Hence, a complete and current food composition data is crucial in order to facilitate the decision making among the policy-maker.

Pitaya or pitahaya is known as dragon fruit due to the scaly structure of the peel. There are three varieties of pitaya namely white-flesh pitaya with yellow peel (*Selenicereus megalathus*), white-flesh pitaya with red peel (*Hylocereus undatus*) and red-flesh pitaya with red peel (*Hylocereus polyrhizus*). Pitaya is a cactus fruits that originated from Central and Northern

South America. Red pitaya (*Hylocereus polyrhizus*) is widely cultivated in Malaysia, Thailand, Vietnam, Australia, Taiwan and some other parts of the world. This fruit survived in the dry tropical climate and can withstand temperature as high as 40°C (Ortiz-hernández and Carrillo-salazar, 2012). It has been introduced to the Malaysian agriculture in 1990's and cultivated in Johor, Perak, Negeri Sembilan, Pahang, Pulau Pinang and Sabah (Department of Agriculture Sarawak, 2013). While in Australia, the fruit is grown in Northern Territory, Queensland, New South Wales and parts of Western Australia.

The fruit of red pitaya (*Hylocereus polyrhizus*) is oval shape, large in size, weighing about 300-600 grams, 32 cm – 35 cm diameter and 13 cm - 15 cm long. The fruit has delicate and sweet flesh with intense red-purple colour of the flesh and peel. It has a lot of small black seeds which are rich in essential fatty acids (Ariffin *et al.*, 2009). The fruits often consumed fresh or make into juices, cordial, jams and ice cream. The pigment that is responsible for the red colour of the fruit is betacyanin (Wybraniec *et al.*, 2001; Stintzing *et al.*, 2002; Wybraniec and Mizrahi, 2002).

Extensive researches have been conducted on the properties of red pitaya pigment (Stintzing *et al.*, 2003; Stintzing *et al.*, 2004) and the antioxidant properties of flesh and peel of red pitaya (Wu *et al.*, 2006; Nurliyana *et al.*, 2010; Omidzadeh *et al.*, 2011). Esquivel *et al.* (2007) found that the betalain compounds including betacyanin contributed

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significantly to the antioxidant properties of red pitaya while other compound only contributed to a minor extent. While several authors (Mohd Adzim Khalili *et al.*, 2006; Ruzainah *et al.*, 2009; Chemah *et al.*, 2011) have conducted proximate analyses of the freeze-dried red pitaya fruits, to our best knowledge there is no publications of proximate analysis of red pitaya juice has been made so far.

Hence, this research was conducted to determine the nutritional composition, minerals content, antioxidant vitamins and phenolic content of red pitaya juice from Malaysia and Australia. In addition, the effects of ethanol concentration on total phenolic, total flavonoid and betacyanin content were investigated. Thus, the present study would give information on the variation of nutritional composition and phenolic content of red pitaya juices in Malaysia and Australia. It is very important for the community to know the nutritional quality of the fruit in order to make an informed choice. Furthermore, the increased available data on nutritional composition of the same fruits from different place confirmed the idea that it is recommended to have guidelines for specific fruits and vegetables consumption for specific country, and not the general recommendation because not all fruits are created equal.

## Materials and Methods

### Materials

All chemicals and reagents used for proximate and spectrophotometric analysis were of analytical grade; gallic acid, aluminium chloride hexahydrate ( $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ ), and rutin from Sigma-Aldrich (MO, USA), absolute ethanol, Folin-Ciocalteu reagent and sodium carbonate were purchased from Merck (Darmstadt, Germany); aluminium chloride hexahydrate were from Fischer Scientific (NH, USA); All chemicals and reagents used for HPLC analysis were from Merck (Darmstadt, Germany) HPLC grade; tetrahydrofuran, ethanol, petroleum ether, acetonitrile, dichloromethane, chloroform, ammonium acetate, metaphosphoric acid, potassium dihydrogen phosphate, sodium sulphate and potassium hydroxide were provided by Sigma-Aldrich (MO, USA).

### Sample preparation

Red pitaya (*Hylocereus polyrhizus*) was obtained from four year old plants at Multi Rich farm in Mantin, Negeri Sembilan, Malaysia. Red pitaya was freshly harvested when reaching full ripening stage, 35-38 days after pollination and immediately transported to the Nutritional Laboratory, Faculty of Medicine and

Health sciences, Universiti Putra Malaysia. The fruits were cleaned, pat dried and weighed. The fruit pulp was cut into small cubes and crushed using macerated blender. The juice was then kept in tight container and was stored in  $-20^\circ\text{C}$  fridge before further analysis. Red pitaya planted in Australia was obtained from the fresh market, Brisbane, Queensland Australia. The fruits were then cleaned, pat dried and weighed. The fruit pulp was squeezed using juice maker. The juice was kept in tight container and then transported to Malaysia by flight in controlled temperature for further analysis. Sample preparation was conducted in reduced light condition in order to minimize the pigment loss.

### Proximate analyses

Red pitaya juices were investigated for nutritional composition by using standard AOAC (1990) method for moisture, ash contents and total dietary fibre. Total available carbohydrate was determined based on Clegg Anthrone's method (Clegg, 1955) while total protein was estimated by using Kjeldahl method (Tee *et al.*, 1996). For total fat, Soxhlet method by Tee *et al.* (1996) was used.

### Determination of minerals content

Minerals content in red pitaya juice was determined according to standard AOAC (1990) method. Briefly, the ashing procedures were conducted. Five millilitres of hydrochloric acid was added into the silica basin to break up the ash and dried over water bath. Then, 2 mL of concentrated hydrochloric acid was added to dissolve the ash and filtered through whatman No. 42 filter paper. After diluted to 100 mL, the sample was read directly on atomic absorption spectrophotometer. All samples were analysed in triplicate and were expressed as mg/100 g FW.

### Antioxidant vitamins

#### Extraction of Vitamin A ( $\beta$ -carotene)

The method for vitamin A extraction was based on Tee *et al.* (1997). Approximately 5 grams of sample was mixed thoroughly with 20 mL of 95% alcohol in a boiling flask. Five millilitres of 100% potassium hydroxide was added slowly. The boiling flask was attached to water-cooled refluxing apparatus and heated on water bath at a reflux rate two drops per second. After 30 minutes reflux, the flask was cooled to room temperature. The hydrolysate was extracted three times with 50 mL of aliquots of hexane. The hexane extract was washed with distilled water until washings are free from alkali and passed through

anhydrous sodium sulphate to dry. The hexane extract was evaporated to dryness and 10 mL of ethanol was added to 10 mL flask. The extract was filtered through 0.45 µm filter.

#### *Extraction of Vitamin C (Ascorbic acid)*

The analysis of vitamin C was conducted based on the method by Abushita *et al.* (1997). The sample (10 g) was mixed with 50 mL of 2% metaphosphoric acid and placed in a conical flask. The mixture was mechanically shaken with orbital shaker for 15 minutes at room temperature. The mixture was then filtered through a Whatman No. 1 filter paper to obtain clear extracts.

#### *High Performance Liquid Chromatography (HPLC) Analysis*

The vitamins were identified and quantified using HPLC system (Agilent 1100, CA, USA) with diode array detector (DAD). The column system consisted of a reversed-phase C18 column (Nova-Pak, 150 mm X 4 mm, 5 µm) from Waters (MA, USA). For the determination of vitamin A, the mobile phase consisted of acetonitrile-methanol-acetyl acetate (88:10:2), operated at a flow rate of 1.2 mL/min with the wavelength was set at 450 nm. The temperature of the column was kept at 22.5°C. Whilst, the determination of vitamin C was using 0.1 M potassium dihydrogen phosphate-methanol (97:3) mobile phase at a flow rate of 1.0 mL/min, with the wavelength was set at 254 nm and the temperature was maintained at 25°C. The prepared mobile phase was degassed using ultrasonic agitation and then filtered under vacuum through a 0.45 µm nylon membrane filter before analysis.

#### *Polyphenolic contents*

##### *Sample extraction*

Red pitaya juice (1 mL) was mixed with 25 mL of deionised water for a few minutes (4%, w/v). The juice mixture was shaken using shaking incubator at 200 rpm for 120 minutes at 50°C and then filtered using buchner funnel to give a coloured solution. The filtration process was repeated for full pigment recovery. The extract was centrifuged at 6000 rpm for 15 minutes at room temperature and supernatant was collected for the determination of total phenolic and total flavonoid content, and betacyanin content.

##### *Total phenolic content (TPC)*

Total phenolic content was carried out using the Folin-Ciocalteu's reagent as described by Singleton and Rossi (1965) with slight modifications. The total

phenolic content was calculated using regression equations from standard curve of gallic acid (0.02–0.08 mg/mL in water). The results were expressed as milligrams of GAE per 100 g of fresh weight. Briefly, red pitaya extract (200 µL) was mixed with 1.5 mL of Folin–Ciocalteu reagent (previously diluted tenfold with distilled water) and allowed to stand at room temperature for 5 min. A 1.5-mL sodium bicarbonate solution (60 g/L) was added to the mixture. The tubes were vortexed, covered with parafilm and allowed to stand for 90 minutes. The absorbance was measured at 750 nm using Secomam's RS232 ultraviolet–visible (UV–vis) spectrophotometer (Cedex, France) after a 90-min incubation at room temperature.

##### *Total flavonoid content (TFC)*

Aluminium trichloride colorimetric method by Quettier-Deleu *et al.* (2000) was used to determine the flavonoid content. One millimetres of red pitaya juice extract solution was added to 1 mL of 2%  $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ . The absorbance was measured 10 minutes later at 430 nm. The result was expressed in mg rutin/100 g fresh weight by comparison with standard rutin treated in the same condition. The standard calibration curve was plotted at 0.02, 0.04, 0.06, 0.08, and 0.10 mg/mL.

##### *Betacyanin content (BC)*

The betacyanin content of fresh extracts was determined by the spectrophotometric method by Wybraniec and Mizrahi (2002). Betacyanin content was expressed as betanin equivalents (mg/100 g of fresh extracts) based on the formula below;

Concentration of betacyanins (mg/100 g fresh weight)

$= A_{538} (\text{MW}) V (\text{DF}) \times 100 / \epsilon \text{LW}$ , where,

$A_{538}$  = absorbance at 538 nm

L = path length, 1.0 cm

DF = dilution factor

V = pigment solution volume (mL)

W = dried pigment weight (g)

For betanin,  $\epsilon$  (molar extinction coefficient) = 65,000 and MW = 550.

#### *Effects of extraction solvent on total phenolic content (TPC), total flavonoid content (TFC) and betacyanin content (BC)*

Red pitaya juice was extracted using dual solvent extraction system whereby ethanol and deionised water was used. Ethanol concentration ranged from 0% (v/v) to 100% (v/v) in the ethanol-water mixture. The optimum ethanol concentration for red pitaya

juice was determined based on the highest total phenolic, total flavonoid and betacyanin content.

### Statistical analysis

Data were presented as mean  $\pm$  standard error of mean (SEM). Statistical analysis was conducted by using SPSS for Windows version 21.0. One-way analysis of variance (ANOVA) with Tukey's test was used to test for significant differences among levels of treatment. Independent-Samples T-Test was used to test for significant difference between the two samples and the significant values were considered at the level of  $p < 0.05$ .

## Results and Discussion

### Nutritional composition

In this study, the proximate composition of red pitaya juice from Malaysia and Australia was investigated. The moisture content, ash, total available carbohydrate, crude fat, crude protein and total available fibre were determined through Standard Operation Procedure (SOP) in the food analysis lab. As shown in Table 1, the juice obtained from red pitaya from Malaysia had  $85.05 \pm 0.11$  g/100 g moisture,  $0.54 \pm 0.01$  g/100 g ash,  $12.97 \pm 0.11$  g/100 g carbohydrate,  $1.45 \pm 0.01$  g/100 g protein, and  $2.65 \pm 0.03$  g/100 g total dietary fibre. On the other hand, the juice from red pitaya cultivated in Australia contained  $89.98 \pm 0.02$  g/100 g moisture,  $1.19 \pm 0.06$  g/100 g ash,  $8.42 \pm 0.03$  g/100 g carbohydrate, and  $0.41 \pm 0.01$  g/100 g protein. In contrast to the previous findings, the present study found slightly higher moisture content ranging from 85% to 89% for red pitaya juice from Malaysia and Australia compared to 82%-85% in the freeze-dried flesh of red pitaya cultivated in Malaysia found by Ruzainah et al. (2009). Furthermore, the moisture content of red pitaya juice from Malaysia was indifferent with the finding obtained by Mohd Adzim Khalili et al. (2006) as the freeze-dried red pitaya had the moisture content of 87%. Besides, the author also indicated that the ash content of 0.70 g is slightly higher than one found in the red pitaya juice from Australia. Interestingly, the crude fibre from the red pitaya juice is about 3.8 times lower than the freeze-dried red pitaya from Malaysia (Mohd Adzim Khalili et al., 2006). However, red pitaya juice from Malaysia is still considered a good source of fibre as compared to red pitaya cultivated in Australia ( $2.65 \pm 0.03$  g/100 g in RPM and no fibre was detected in RPA, respectively). High moisture content of food is related to high perishability (Adelekeg and Abiodun, 2010), but the large volume of water and fibre in fruits can reduce their energy content and therefore are low in

Table 1. Nutritional composition, minerals and vitamins content red of pitaya from Malaysia and Australia

Analyses	Values	
	RPM	RPA
Moisture (g/100 g FW)	$85.05 \pm 0.11^b$	$89.98 \pm 0.02^a$
Ash (g/100 g FW)	$0.54 \pm 0.01^b$	$1.19 \pm 0.06^a$
Carbohydrate (g/100 g FW)	$12.97 \pm 0.11^a$	$8.42 \pm 0.03^b$
Protein (g/100 g FW)	$1.45 \pm 0.01^a$	$0.41 \pm 0.01^b$
Fat (g/100 g FW)	ND	ND
Total Dietary Fibre (g/100 g FW)	$2.65 \pm 0.03$	ND
Energy (kcal/100 g) †	$62.95 \pm 0.45^a$	$35.36 \pm 0.15^b$
Iron (mg/100 g FW)	$0.30 \pm 0.01^a$	$0.03 \pm 0.001^b$
Magnesium (mg/100 g FW)	$26.40 \pm 0.07^a$	$13.7 \pm 0.11^b$
Potassium (mg/100 g FW)	$158.29 \pm 1.09^b$	$437.35 \pm 0.54^a$
Sodium (mg/100 g FW)	$35.63 \pm 0.14^a$	$14.30 \pm 0.35^b$
Zinc (mg/100 g FW)	$0.40 \pm 0.02^a$	$0.09 \pm 0.004^b$
Calcium (mg/100 g FW)	$6.72 \pm 0.02^a$	$1.55 \pm 0.02^b$
Vitamin A ( $\mu$ g/100 g FW)	$85.22 \pm 3.21^b$	$890.8 \pm 14.30^a$
Vitamin C ( $\mu$ g/100 g FW)	$24.66 \pm 0.03^b$	$30.21 \pm 0.34^a$

Data are presented as mean  $\pm$  SEM of triplicate measurements (n = 3). Means with different letters in the same row are significantly different at the level of  $p < 0.05$ . RPM, red pitaya from Malaysia; RPA, red pitaya from Australia; ND, not detected; FW, fresh weight. † The energy content was determined from the amounts of protein, fat, and carbohydrate in the foods using energy conversion factors of 4 kcal for carbohydrate, 4 kcal for protein, 9 kcal for fat and 2 kcal for fibre.

energy density (Rodri et al., 2010). A cross-sectional survey of adults in United States found that high consumption of fruits reduced energy density values and hence resulted in lowest prevalence of obesity (Ledikwe et al., 2006) suggested the role of fruits and vegetables in weight and fat loss management (Whigham et al., 2012).

The protein content found in the present study was higher ( $1.45$  g and  $0.41$  g for red pitaya from Malaysia and Australia, respectively) compared to the previous studies which were  $0.159$ - $0.229$  g (Ruzainah et al., 2009) and  $0.16$  g protein (Mohd Adzim Khalili et al., 2006). In contrast to Ruzainah et al. (2009) and Mohd Adzim Khalili et al. (2006) that detected fat content up to  $0.61$  g in the red pitaya, the present study did not detected fat from the red pitaya juice. Similarly, Chemah et al. (2011) reported that the fat from pulp of red pitaya was only  $0.1\%$  compared to  $22.8\%$  from the seeds. The reason for this finding could lie on the sample preparation process as it can influence the recovery of fat from the sample since the seeds of red pitaya contained significant amount fatty acids (Ariffin et al., 2009). Furthermore, previous researches used freeze-dried sample and freeze-drying method has shown to preserve the labile analytes and break the plant cell membrane, hence increase the extraction efficiency as compared to extracting from fresh materials (Nurul and Asmah, 2012). Moreover, the carbohydrate content was higher in the red pitaya from Malaysia ( $12.97$  g), than the red pitaya from Australia ( $8.42$  g). To the best of our knowledge, this is the first time energy intake of the red pitaya juice was reported. It is shown here that red pitaya juice from Malaysia provided twice the total calorie intake compared to red pitaya juice from Australia. The difference was expected as the composition of macronutrients of red pitaya juice from both countries was significantly different.

### Minerals content

Mineral composition for red pitaya in the present work was not indicated by the ash content which is contradicts with the findings from previous research (Hasnah *et al.*, 2009). The ash content of red pitaya juice from Australia was significantly higher ( $p < 0.05$ ) than red pitaya from Malaysia. However, it appeared that the concentration of macroelements such as magnesium, sodium, and calcium and the concentration of trace elements iron and zinc were significantly higher ( $p < 0.05$ ) in the red pitaya juice from Malaysia (table 1). Furthermore, the red pitaya from Australia only showed higher amount of potassium. Nevertheless, Mohd Adzim Khalili *et al.* (2006) found low amount of potassium (56.96 mg) which is about 2.8 (158.29 mg) and 7.7 (437.35 mg) times lower than in RPM and RPA respectively found in the present study. On the other hand, the authors (Mohd Adzim Khalili *et al.*, 2006) reported that the amount of iron (3.40 mg), magnesium (28.30 mg), sodium (35.63 mg), and zinc (13.87 mg) contents were higher as compared to the present study (iron, 0.30 mg and 0.03 mg in RPM and RPA; magnesium, 26.40 mg and 13.7 for RPM and RPA; sodium, 35.63 mg and 14.30 for RPM and RPA; zinc, 0.40 mg and 0.09 mg for RPM and RPA respectively). Moreover, calcium (6.72 mg) content in red pitaya juice from Malaysia was higher than the previous study (5.70 mg) (Mohd Adzim Khalili *et al.*, 2006). This implied that red pitaya contain reasonable amount of essential minerals that may act as supplementation into one's diet to meet the daily requirement of these elements. High mineral content in fruits are very crucial for maintaining healthy body. For example, red pitaya may assist in reducing risk for hypertension as it contained high potassium content and the ratio of sodium and potassium of less than one can decrease blood pressure (Akubugwo *et al.*, 2007).

### Antioxidant vitamins ( $\beta$ -carotene and ascorbic acid)

Red pitaya was also a good source of vitamin C as reported by Ruzainah *et al.* (2009) who found 8-9 mg of vitamin C in freeze-dried red pitaya flesh and a fluctuating amount in the stem of red pitaya ranged from 63.71-132.95 mg. The present study found only appreciable amount of vitamin C in red pitaya juice (24.66  $\mu\text{g}/100\text{ g}$  - 30.21  $\mu\text{g}/100\text{ g}$  in RPM and RPA respectively). Vitamin C (ascorbic acid) is easily oxidized and destroyed during food preparation and processing. It is postulated that vitamin C in the present study was loss during the preparation of the juice. Besides, the freeze drying of fruits in the previous study resulted in the preservation of the bioactive compounds in the samples (Tekel *et al.*,

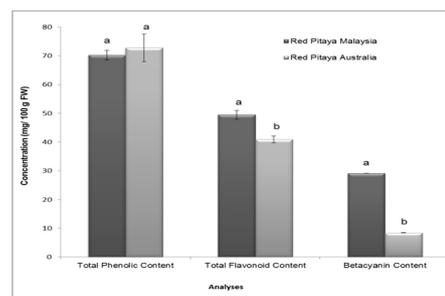


Figure 1. Total phenolic content, total flavonoid content and betacyanin contents of red pitaya from Malaysia and Australia. Data are presented as mean  $\pm$  standard error of mean of triplicate measurements ( $n = 3$ ). Different letters in the same column are significantly different at the level of  $p < 0.05$ .

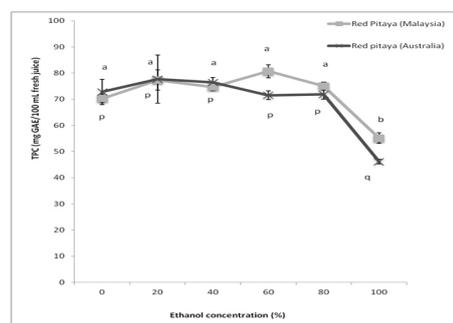


Figure 2. Effect of ethanol concentration on TPC from red pitaya from Malaysia and Australia. Data are presented as mean  $\pm$  standard error of mean of triplicate measurement ( $n = 3$ ). Values marked with different lower case letters (a-e, p-u) are significantly different at the level of  $p < 0.05$ .

1999) and caused insignificant loss to the antioxidant components (Böhm *et al.*, 2006). Vitamin A was extremely high in red pitaya juice from Australia (890.8  $\mu\text{g}/100\text{ g}$ ) compared to red pitaya juice from Malaysia (85.22  $\mu\text{g}/100\text{ g}$ ) and it was comparable with vitamin A content in fresh mango (989  $\mu\text{g}/100\text{ g}$ ), fresh peach (787  $\mu\text{g}/100\text{ g}$ ) and fresh pineapple (937  $\mu\text{g}/100\text{ g}$ ) (Tee *et al.*, 1997).

### Phenolic content

Figure 1 revealed that no significant difference ( $p > 0.05$ ) between TPC of the red pitaya juice from Malaysia (70.24  $\pm$  1.65 mg GAE/100 g FW) and Australia (72.80  $\pm$  4.80 mg GAE/100 g FW). On the other hand, TFC of the red pitaya juice from Malaysia (49.49  $\pm$  1.60 mg GAE/100 g FW) was significantly higher ( $p < 0.05$ ) than red pitaya juice from Australia (40.93  $\pm$  1.19 mg GAE/100 g FW). Likewise, BC of the red pitaya juice from Malaysia (29.19  $\pm$  0.01 mg/100 g fresh weight) was approximately four times higher than the red pitaya juice from Australia (8.43  $\pm$  0.01 mg/100 g fresh weight) (Figure 1).

Besides, the effect of dual extraction solvent on TPC, TFC, and BC was investigated by modifying the ratio between water and ethanol. It had been

documented previously that the different composition of water and ethanol, methanol or acetone could increase the extraction efficiency (Yu *et al.*, 2002; Alothman *et al.*, 2009). A current research by Brahmi *et al.* (2012) pointed out that a diverse amount of phenolic compounds can be extracted when using the different polarity of extracting solvents. As shown in Figure 2, the highest TPC was achieved at 20% (v/v) ethanol for the red pitaya juice from Australia whereas 60% (v/v) ethanol produced highest TPC for the red pitaya juice from Malaysia. Therefore, this finding indicated that the red pitaya juice from Malaysia contained more lipid soluble or lipophilic phenolic compounds. However, no fat was detected from the juice of red pitaya in the present study. It is important to highlight that the extraction methods for the determination of fat and phenolic compounds were different. The high water content in the sample may interfere with the penetration of organic solvent into the sample which in turn resulted in inefficient extraction during the determination of fat. Also, the oxidation process may cause the loss of fat in the sample that already has very low amount of fat. In contrast, the determination of TPC using colorimetric method is depending upon the amount of phenolic hydroxyl groups in the sample extract. As the number of hydroxyl group present decreases, the polarity of the phenolic compound decreases (Lattanzio *et al.*, 2006). Thus, it can be hypothesized that the lipid and water soluble phenolic compounds can be easily extracted using the mixture of water and ethanol.

In principle, phytochemicals will be only extracted from the plant cells wall by the solvent that have similar polarities. The present study demonstrated that dual-solvent system was more effective than that to mono-solvent system in extracting phenolic and flavonoid compounds. This finding was in agreement with previous study as reported by Wang *et al.* (2013). The study pointed out that TPC of pomegranate leaves increased when using 50% ethanol as the extraction solvent compared by using water alone. Result from the present study showed that the red pitaya from Malaysia was more soluble in weak polarity extraction medium in contrast to the red pitaya from Australia that contained more polar phenolic compounds. The reverse trend was observed for total flavonoid content suggested that TFC of red pitaya from Australia was more soluble in moderate polarity extraction medium while red pitaya from Malaysia contained more polar compounds. Despite of that, the highest recovery of betacyanin compounds in the red pitaya from Malaysia was exhibited at 0% ethanol indicated the presence of only water soluble betacyanin pigments.

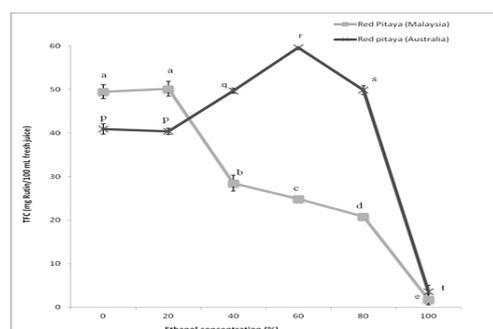


Figure 3. Effect of ethanol concentration on TFC from red pitaya from Malaysia and Australia. Data are presented as mean  $\pm$  standard error of mean of triplicate measurement ( $n = 3$ ). Values marked with different lower case letters (a-e, p-u) are significantly different at the level of  $p < 0.05$ .

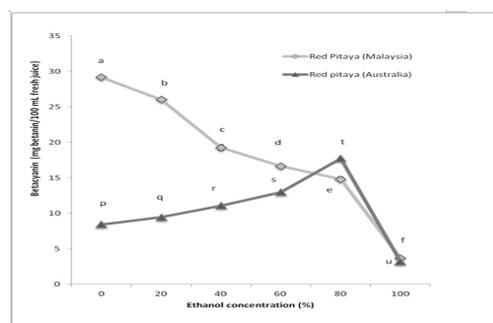


Figure 4. Effect of ethanol concentration on BC from red pitaya from Malaysia and Australia. Data are presented as mean  $\pm$  standard error of mean of triplicate measurement ( $n = 3$ ). Values marked with different lower case letters (a-e, p-u) are significantly different at the level of  $p < 0.05$ .

The reduced extraction of phenolic and flavonoid compounds at 100% (v/v) ethanol could be due to the interference from the extraction of lipid constituents as observed in a study on phenolic contents in wheat bran by Wang *et al.* (2008). Besides, the maximum TFC yielded at 20% ethanol for the red pitaya juice from Malaysia and 60% ethanol for the red pitaya juice from Australia (Figure 3), suggested that the red pitaya juice from Malaysia had more hydrophilic flavonoid compounds. In contrast, the highest BC for the red pitaya juice from Malaysia was at 0% ethanol while BC for the red pitaya juice from Australia was highest when extracted with 80% ethanol but it levels reduced drastically with use of 100% ethanol.

In this work, the discrepancy of nutritional composition, minerals, vitamins and phenolic contents in red pitaya suggested that geographical regions had strong influence on the phytochemical properties of the plant. It is strongly believed that the environmental factors including climate and soil had significant effects on phenolic and antioxidant compounds of blueberry (Connor *et al.*, 2002), red raspberry (Antonien *et al.*, 2005) dabai fruits (Chew *et al.*, 2011), fresh and pickled papaya (Nurul and Asmah, 2012). Furthermore, transportation

and storage may also affect vitamins and minerals content of fruits (Jagdish *et al.*, 2007) since red pitaya was transported to Malaysia for the analysis. Another important point to consider is the timing of samples collection. Gadže *et al.* (2012) found that the accumulation of phenolic compounds and pigment like anthocyanin and betacyanins were higher during a colder season. In fact, the biosynthesis of these bioactive compounds is a time dependent regulated process (Jiang *et al.*, 2013). Thus, different climate condition between Malaysia and Australia could explain the differences in nutrient and non-nutrient compositions of red pitaya cultivated in both countries.

## Conclusion

In conclusion, this present work clearly demonstrated the variability in the nutritional composition and phenolic content of red pitaya juices from Malaysia and Australia. This finding clearly indicated promising perspectives for the exploitation of other fruits with considerable levels of nutrients and antioxidant compounds like polyphenols. Red pitaya has been cultivated in Malaysia and consumed by Malaysian for decades and but have not been included in Nutritional Composition of Malaysian Foods. Thus, it is suggested to include the data to assist in making informed choice to the community. The selection of samples from the two countries was based on convenience sampling. Hence, the interpretation of result warrants further consideration as the data obtained will not represent the whole plant being analysed. Despite of that, the scientific assessment of chemical constituents of red pitaya grown in different countries add valuable information to current knowledge on the nutritional composition and phytochemical properties of red pitaya in different geographical region.

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