

Determination of pitaya seeds as a natural antioxidant and source of essential fatty acids

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Abstract: The antioxidant capacity and total phenolic content of seeds of three types of pitaya namely *Hylocereus polyrhizus*, *Hylocereus undatus* and *Selenicereus megalanthus* and the fatty acid content of pitaya seeds oil were examined in this study. The ethanolic extracts of *H. polyrhizus* seeds showed significantly high ($p < 0.05$) total phenolic (43.9 mg GAE/100g dry weight) and flavonoid (50.8 mg CAE /100g dry weight) contents as compared to *H. undatus* and *S. megalanthus* and its aqueous extracts. DPPH assay showed similar trend showing that *H. polyrhizus* seeds had significantly higher ($p < 0.05$) scavenging capacities (46.6%). FRAP test also indicated that the ethanolic extracts of *H. polyrhizus* seeds had significantly higher ($p < 0.05$) reducing capacity (59.1mg Trolox/100g dry weight). The main fatty acids of pitaya seeds oil were C16:0, C18:0, C18:1, C18:2 with an exceptional high level of linoleic acid, up to 660 g/kg for *S. megalanthus*, 540 g/kg for *H. undatus* and 480 g/kg for *H. polyrhizus*.

Keyword: antioxidant, pitaya seeds, essential fatty acid, *H.polyrhizus*, *H.undatus*, *S.megalanthus*

Introduction

Several million tons of agri-food solid wastes are produced annually and disposed through various methods (Isci and Demirer, 2007). Investigations have been focusing on the re-use of waste materials into food ingredients, bio-fuels, and other value-added application (Makris *et al.*, 2007; Tsai *et al.*, 2007). The usage of residual sources of fruits and vegetables as functional foods has also been discussed extensively (Laufenberg *et al.*, 2003; Schieber *et al.*, 2001). The by-products of fruit and vegetables processing such as peels and seeds have the potential as good antioxidant sources (Laufenberg *et al.*, 2003; Moure *et al.*, 2001; Chemah *et al.*, 2008). Antioxidants in product development of foods, cosmetic and pharmaceutical industries have been proven to be of importance because of similar functionality requirements for the products (Ku and Mun, 2007). There are several studies reported on the reuse of seeds for other industrial wastage such as from wine processing waste (Ku and Mun, 2007); from fruit seeds (Soong and Barlow, 2004); cactus fruit seeds (Chang *et al.*, 2008) and mango seed waste (Abdalla *et al.*, 2007).

Pitaya (*Hylocereus undatus* and *Hylocereus polyrhizus*) or locally known as dragonfruit is a new

crop being cultivated commercially in Malaysia. However it has been grown in Vietnam for at least 100 years after been introduced by the French (Mizrahi *et al.*, 1997). This climbing cactus is known as red pitaya in Latin America, with medium-large berry bearing large green or red scales (Nerd and Mizrahi, 1997) which resembles a dragon, hence the name Thanh Long or green dragon in Vietnam (Hoa *et al.*, 2006). The peel is usually red, and pulp varies from purple red to white. The pulp is delicate and juice containing many small soft seed (Nerd *et al.*, 1999). These cactus plants are grown openly in tropical areas with good water flow and lots of sun.

Yellow pitaya (*Selenicereus megalanthus*) is a related cactus originated in the northern part of South America (Mizrahi *et al.*, 1997) is also being cultivated in Columbia and Israel (Nerd and Mizrahi, 1999). The fruit is a medium-sized oblong berry with a yellow peel bearing tubercles and thorns that are shed during ripening. The pulp is white and delicate and contains numerous small digestible black seeds (Nerd and Mizrahi, 1997). Yellow pitaya, had a different duration of fruit development which depends on seasonal temperatures, and the fruits reach the optimal flavour close to full colour stage (Nerd and Mizrahi, 1998). It is a non climateric fruit and has the best quality when picked close to the full colour

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stage (Nerd and Mizrahi, 1998).

Pitaya seeds which are the by-products of juice and wine processing were usually discarded. It is the objective of this study to investigate the content of pitaya seeds and the potential of the seeds as a source of antioxidant. To the best of our knowledge no studies has ever been conducted on the potential of pitaya seeds as an antioxidant and essential fatty acid.

Materials and Methods

Production of seed powders

Seeds from three types of pitaya, *Hylocereus undatus*, *Hylocereus polyrhizus* and *Selenicereus megalanthus* were manually separated from the pitaya pulp in the lab. The seeds were cleaned and washed under running water until all the pulp removed. Seeds were later dried in the oven at 40°C overnight and kept in a dessicator until further analysis.

Proximate analysis of pitaya seed

The proximate analysis was determined according to AOAC (1990) methods. Carbohydrate was determined by difference.

Pitaya seeds extraction

Pitaya seeds were finely ground before being extracted with aqueous and 50% ethanol. Half gram (0.5g) of seeds sample were mixed with 10 ml of water and 50% ethanol before homogenized at 24000 rpm (Ultra-Turrax, IKA, Germany) for 1 min and centrifuged for 20 minutes at 4000 g (Kubota Model 2420) at room temperature. The extracts were obtained from 2 batches of seeds prepared. Each batch contained 3 replicates.

Total phenolic content

Total phenolic content were determined using Folin-Ciocalteu reagent, modification method by Wolfe et al. (2003). Gallic acid was used as standard and the concentration of total phenolic compounds in the extracts were calculated by standard curve interpolation. Results were reported as mg gallic acid equivalent/100 g dried sample.

Determination of total flavonoid content

Flavonoid were measured using a method by Liu and Zhu (2007) with slight modification. Catechin was used as standard and total flavonoid in the extracts were calculated by standard curve interpolation. Results were expressed as mg catechin/100 g dry weight.

Antioxidant capacity assay

DPPH assay

Free radical scavenging activity were measured by the 2,2- diphenyl-1-picrylhydrazil (DPPH) according to a modified method by Wu *et al.* (2006). Sample extracts (100ul) were added into 3.9ml of DPPH reagent (prepared with 24mg of DPPH/L of methanol). The percentage of DPPH scavenging activity is expressed by the following formula,

DPPH

$$\text{inhibition} = \frac{[(\text{Initial absorbance} - \text{sample absorbance}) \times 100]}{\text{Initial absorbance}}$$

FRAP assay

FRAP method were according to Benzie and Strain (1996) which measures the ferric reducing ability of plasma (FRAP). The method is based on the reduction of a ferric 2,4,6-tripyridyl-s-triazine complex (Fe³⁺-TPTZ) to the ferrous form (Fe²⁺-TPTZ). The stock solutions included 300mM acetate buffer (3.1 g C₂H₃NaO₂·3H₂O) and 16mL C₂H₄O₂), pH 3.6, 10mM TPTZ (2, 4, 6-tripyridyl-s-triazine) solution in 40mM HCl, and 20mM FeCl₃·6H₂O solution. The fresh working solution was prepared by mixing 25mL acetate buffer, 2.5mL TPTZ solution, and 2.5mL FeCl₃·6H₂O solution and then warmed at 37°C before using. The reagent were added into 100ul sample extracts. The reduced form of blue colour were read at 593nm after 30 minutes. Trolox was used as standard and ferric reducing power of the extracts were calculated by standard curve interpolation. Results were expressed as mg Trolox equivalent/100 g dry weight.

Fatty acid methylation of pitaya seeds oil

Fatty acid composition (FAC) of the samples was analyzed as fatty acid methyl esters (FAMES), which were prepared according to IUPAC 2.301 (1987). FAMES were then analyzed using gas chromatography (Waters) with FID detector. Column used was 60 m x 0.25 mm film 0.25 um (J&W Scientific DB 23). The injection temperature 130°C with flame ionizing detector temperature at 220°C. Nitrogen carrier gas was used at a rate of 0.53ml/min.

Statistical methods

Data were analyzed statistically using SAS version 6.12. All samples were prepared and analyzed in triplicates except for FAME which is in duplicate. To verify the statistical significance of all parameters, the values of means ± SD were calculated. Analysis of variance (ANOVA) was used to compare several groups. Probability value of p < 0.05 was adopted as the criteria for significance differences.

Results and Discussion

Proximate composition

Seeds constitute about 2-5 % dry weight of the whole fruit and are usually discarded in juice or wine processing. The proximate composition of pitaya seeds (*Hylocereus polyrhizus*, *Hylocereus undatus* and *Selenicereus megalanthus*) are showed in Table 1.

The moisture content of the seeds were between 6.9% to 7.2%. The results in Table 1 showed that protein and fat were high. Protein content was between 21.5 to 26.6% dry weight. These result are higher than the protein content of another cactus namely *Opuntia ficus indica* which contained 1.5 % dry weight (Habibi *et al.*, 2008). However, the protein range were suitable for seeds of legumes type such as soybean which has 24.5 % of protein (Jeff-Agboola and Oguntuase, 2006), 39.5% in chinese bottle *Lagenaria sciceraria* (Olaofe *et al.*, 2009) and 35 % in *Piliostigma thonningii* seeds (Jimoh and

seeds with 14-16% (Kamer *et al.*, 1985). Apple seeds contained very high protein ~34% content however grape seeds was low with 7 to 10%. Ash content of pitaya seeds in this study resulted with 3.1 to 6.1 % and was between the apple seeds ~4.1% as reported in Yu *et al.* (2007) and grape seeds with 2.2% (Kamer *et al.*, 1985).

Phenolic and flavonoid content

Phenolic and flavonoid content of pitaya seeds are showed in Table 2. Polyphenolic are found abundant in fruits and vegetables and it is one of the major parameters to determine the significance of the type of fruits and vegetables. Although it is a general method, Folin-Ciocalteu assay is one of the most commonly used method to determine total phenolic in fruits and vegetables.

Ethanol extracts of *H.polyrhizus* seeds showed significantly higher ($p < 0.05$) phenolic content (43.9 mg GAE/100g dry weight). The seed of *H.polyrhizus* had higher phenolic ($p < 0.05$) content than the other

Table 1. Proximate composition of pitaya seeds (g/100 g)

Parameter	<i>H. polyrhizus</i>	<i>H. undatus</i>	<i>S. megalanthus</i>
Protein	26.3 ± 0.2 ^b	23.1 ± 0.1 ^c	28.6 ± 0.4 ^a
Oil	22.8 ± 0.5 ^b	27.5 ± 1.0 ^a	18.8 ± 0.8 ^c
Ash	6.1 ± 0.0 ^a	3.1 ± 0.1 ^c	3.8 ± 0.1 ^b
Carbohydrate	44.8 ± 0.3 ^b	46.3 ± 1.1 ^b	48.7 ± 1.1 ^a

Each value is mean of three determinations (n=3) $p < 0.05$.

Carbohydrate were determined by difference.

Olidiji, 2005).

The oil content of *H.polyrhizus*, *H.undatus* and *S.megalanthus* in this study was 22.8%, 27.5% and 18.8% respectively. These values were higher than the crude oil from another type of cactus i.e. *Opuntia ficus* which contained 10.9 to 11.1% oil (Ennouri *et al.*, 2006). Additionally, Habibi *et al.* (2008) found the fat/wax content is 8% in *Opuntia ficus indica*. As for the ash content in pitaya seeds, the results were in the range of 2.9 to 5.7% and this result is in agreement with Habibi *et al.* (2008).

In summary the proximate composition of pitaya seeds were comparable to the apple seeds with high protein and oil contents. Pitaya seeds contained oil at the range between 18.8 - 27.5% as compared to apple seeds ~27.7% (Yu *et al.*, 2007) but higher than grape

two type of seeds. Generally the ethanolic extracts showed better extraction recovery. The recovery of polyphenols from plant materials is influenced by the solubility of the phenolic compounds in the solvent used for the extraction process (Alothman *et al.*, 2009). In addition, the polarity of the solvent will play a key role in increasing phenolic solubility (Naczka and Shahidi, 2006). However when compared with seeds of another cactus *Opuntia dillenii* Haw, the latter had almost 10 fold higher phenolic and flavonoid content than the pitayas (Chang *et al.*, 2008). The phenolic contents of pitaya seeds were comparable to the caneberry seed composition (44.6 - 58.2 mg GAE/g seeds) as reported by Bushman *et al.* (2004). Fruits and vegetable contained phenolics and flavonoids, however its content varies between

types and varieties.

Antioxidant activity of pitaya seeds extracts

The antioxidant assays as determine by DPPH scavenging abilities of the extracts showed that *H.polyrhizus* ethanolic seed extracts had higher capacity ($p<0.05$). Ethanolic extracts showed significantly higher DPPH scavenging capacity compared to the water extracts. The results indicated that the antioxidant effectiveness decreases in the following order *H.polyrhizus* seeds ethanolic extracts (46.6%), *H.undatus* (44.5%) and *S.megalanthus* (44.6%).

The FRAP results showed similar trend, the ethanolic extracts had better antioxidant capacity than the water extracts. *H. polyrhizus* seeds resulted with 59.1mg Trolox equivalent/100g dry weight of seeds showed significantly higher ($p<0.05$) antioxidant capacity as compared to the other two types of pitaya seeds. Grape seeds exhibited high antioxidant capacity when tested under FRAP assay (Guo *et al.*, 2003). While, Yilmaz and Toledo (2006) found that the grape seeds exhibited better antioxidant capacity than the peel. The study by Yilmaz and Toledo (2006) also found that aqueous mixtures of either ethanol, methanol or acetone were better than a monocomponent solvent for the extraction of phenolics from the grape seeds.

Our results were in agreement with Yilmaz and Toledo (2006), which reported that the 50% ethanol extracts showed higher antioxidant capacity.

There are several methods being used to measure antioxidant capacity of fruits such as direct competition methods and indirect methods (Roginsky

and Lissi, 2005). But, there are also many different antioxidants contained in fruits and thus were difficult to measure each antioxidant individually (Guo *et al.*, 2003). However, DPPH and FRAP assay were common, simple to standardized and reproducible (Thaipong *et al.*, 2006). Eventhough the method used is the same in other study, the results sometimes could not correlate, therefore, difficult to compare between study (Guo *et al.*, 2003).

Fatty acid composition of pitaya seeds

Pitaya seeds oil is rich in linoleic ($C_{18:2}$) and oleic ($C_{18:1}$) acids. *S. megalanthus* showed significantly high ($p<0.05$) linoleic acid (65.4%) content as compared to *H. undatus* (53.8%) and *H. polyrhizus* (48.7%). Pitaya seeds contained high linoleic acid when compared to caneberry seeds which contained only 54.2% in the red raspberry seeds and 62.7% for marion blackberry (Bushman *et al.*, 2004). Results from this study showed that pitaya seeds contained higher oleic acid when compared to Bushman *et al.* (2004). Oleic acid in *H. polyrhizus* (25.5%) were significantly higher ($p<0.05$) than the two types of pitaya seeds, 23.3 % for *H. undatus* and 13.92% for *S. megalanthus*. Results showed that the lipid pattern of pitaya seeds oil are comparable to that of cactus pear seed oil (Ramadan and Morsel, 2003; Ennouri *et al.*, 2006); also in sunflower and grapeseed seed oils (Tan and Yaacob, 2000).

Linoleic acid is a polyunsaturated omega-6 fatty acid and it is essential in human diet. Linoleic acid consumption were important to counter the cholesterol increase effect of the carbon 12-16 saturated fatty acid. Although pitaya seeds contained lauric, myristic and

Table 2. Phenolic and flavonoid compounds in water and ethanol (50%) extracts of three types of pitaya seeds

Pitaya	Assay			
	Total phenolic (mg GAE/100g sample)	Flavonoid (mg CAE/100g sample)	DPPH (%)	FRAP (mg Trolox/100g sample)
Ethanol extracts				
<i>H. polyrhizus</i>	43.9 ^a	50.8 ^a	46.6 ^a	59.1 ^a
<i>H. undatus</i>	38.0 ^c	42.2 ^b	44.5 ^b	49.0 ^b
<i>S. megalanthus</i>	40.6 ^b	43.2 ^b	44.6 ^b	47.6 ^b
Water extracts				
<i>H. polyrhizus</i>	33.7 ^d	19.7 ^d	27.6 ^c	32.5 ^c
<i>H. undatus</i>	33.0 ^{de}	30.1 ^c	27.3 ^c	34.3 ^c
<i>S. megalanthus</i>	31.5 ^e	17.0 ^d	23.2 ^d	24.9 ^d

Each value is mean of triplicate determination (n=3)

Alphabet within the same column were significantly difference ($p<0.05$)

Table 3. Fatty acid composition of three types of pitaya seeds oil (g/100g of total fatty acid)

Fatty acid (%)	<i>Hylocereus undatus</i>	<i>Hylocereus polyrhizus</i>	<i>Selenicereus megalanthus</i>
Lauric C _{12:0}	0.0 ^{eA}	0.0 ^{gA}	0.0 ^{dA}
Myristic C _{14:0}	0.2 ^{eAB}	0.2 ^{gA}	0.1 ^{dB}
Palmitic C _{16:0}	13.7 ^{cb}	15.9 ^{ca}	14.4 ^{baB}
Palmitoleic C _{16:1}	0.6 ^{eB}	0.8 ^{fA}	0.4 ^{dC}
Stearic C _{18:0}	4.7 ^{dA}	4.6 ^{dA}	2.2 ^{cb}
Oleic C _{18:1}	23.3 ^{bA}	25.5 ^{bA}	13.9 ^{bb}
Linoleic C _{18:2}	53.8 ^{aB}	48.7 ^{aB}	65.4 ^{aA}
Arachidic C _{20:0}	1.2 ^{edA}	1.4 ^{eA}	0.8 ^{dB}
Behenic C _{22:0}	1.2 ^{edA}	1.4 ^{eA}	1.3 ^{cdA}

Each value is the mean of replicate (n=2). Different letter (small case) within the same column are significantly different (p<0.05). Different capital letter within the same row are significantly different.

palmitic acid (C₁₂-C₁₆) which were believe to have cholesterol-raising potential but the raise of these saturated fatty acid also will raise plasma levels of the protective high density lipoprotein which can act as buffer to counter react the cholesterol (NCCFN, 2005). Dietary linoleic acid level of 3-7% kcal is recommended for Malaysian population (NCCFN, 2005).

Conclusions

Pitaya seeds have potential to be developed as functional food. It has good antioxidant capacity and its fatty acid were of good nature oil. The linoleic acid in the pitaya seeds were as high as 660 g/kg (*S. megalanthus*), 540 g/kg (*H. undatus*) and 480 g/kg (*H. polyrhizus*). Further work on pitaya seeds such as the isolation of antioxidant components could provide information on its potential benefit towards animal and human health.

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