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Chemical composition and DSC thermal properties of two species of Hylocereus cacti seed oil: Hylocereus undatus and Hylocereus polyrhizus

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1. Introduction

The fruit of cacti, known as pitaya or dragon fruit, is a segmented, vine-like crawling cactus with aerial roots. Being an epiphyte, it clings to its support and can obtain nutrients from cracks where organic material concentrates. The fleshy succulent stems are three sided (occasionally four or five) and lobed along the ridges, which have small swellings equipped with short spines. Pitaya has already received worldwide recognition as an ornamental plant for its large, scented, night-blooming flowers. Its fame is now spreading throughout the world for its fruit, especially in Asian countries such as Vietnam, Taiwan, Malaysia, and the Philippines [\(Mizrahi, Nerd, & Nobel, 1997](#page-4-0)). Pitaya comes in a number of varieties; three varieties that have been commercialised are Hylocereus undatus (Red Pitaya), which has red-skinned fruit with white flesh, Hylocereus polyrhizus, which has red-skinned fruit with red flesh, and Hylocereus megalanthus (Yellow Pitaya), which has yellow-skinned fruit with white flesh ([Arcadio, 1986; Barbeau,](#page-4-0) [1990\)](#page-4-0). In Malaysia, about 927.4 ha have been established for planting of pitaya for the local and export markets ([Cheah & Zulkarnain,](#page-4-0) [2008\)](#page-4-0), and the red-flesh pitaya farm size is increasing substantially due to a high demand. The red-flesh pitaya has recently drawn much attention from growers worldwide, not only because of its red–purple colour and economic value as a food product, but also

ABSTRACT

Two types of pitaya (Hylocereus cacti) seeds (Hylocereus undatus and Hylocereus polyrhizus) were investigated in this study. The fatty acid, phenolic, tocopherol, and sterol contents of the extracted seed oil were analysed. The results showed that the pitaya seeds contained a high amount of oil (18.33–28.37%). The three major fatty acids in the H. undatus seed oil (WFSO) and H. polyrhizus seed oil (RFSO) were linoleic, oleic, and palmitic acids. The total tocopherol contents in the WFSO and RFSO were 36.70 and 43.50 mg/ 100 g, respectively. The phytosterol compounds identified in the WFSO and RFSO were cholesterol, campesterol, stigmasterol, and b-sitosterol. Seven phenolic acid compounds were identified in the WFSO and RFSO, namely, gallic, vanillic, syringic, protocatechuic, p-hydroxybenzoic, p-coumaric, and caffeic acids. WFSO and RFSO can be differentiated by their T_{off} and T_{on} values in the DSC thermal curves. This study reveals that pitaya seed oil has a high level of functional lipids and can be used as a new source of essential oil.

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for its antioxidative activity from the betacyanin contents [\(Wybra](#page-5-0)[niec & Mizrahi, 2002](#page-5-0)). In cacti, the most important fruit pigments are betacyanins and betaxanthins [\(Wybraniec et al., 2001\)](#page-5-0). Betalains, composed of red–violet betacyanin and yellow betaxanthins, are water-soluble pigments that provide colours in flowers and fruits.

Efforts have been made to study the chemistry of betalains in H. polyrhizus ([Stintzing, Conrad, Klaiber, Beifuss, & Carle, 2004;](#page-4-0) [Wybraniec & Mizrahi, 2002; Wybraniec et al., 2001](#page-4-0)). Recently, [Arif](#page-4-0)[fin et al. \(2009\)](#page-4-0) profiled the fatty acid composition of pitaya seed oils. However, there is little information available on the valuable chemical compounds present in pitaya seed oil. The aim of this study was to characterise the functional chemical components of H. undatus seed oil (WFSO) and H. polyrhizus seed oil (RFSO). As a by-product of pitaya juice manufacturing, the tiny black seeds of the pitaya could be utilised as a potential new source of specialty oil.

2. Materials and methods

2.1. Chemicals

A set of four tocopherol species (α , β , γ , and δ -T) was purchased from Merck (Darmstadt, Germany). All of the standards were purchased from Sigma Chemical Co. (St. Louis, MO, USA). The other chemicals used were of analytical or HPLC grade (Merck, Darmstadt, Germany).

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2.2. Sample preparation

Fresh and mature H. undatus and H. polyrhizus fruits were obtained from a local fruit supplier, Serdang, Malaysia. The fruits were washed under running tap water and hand peeled. Seed separation was achieved using a sieving machine (''Bonina 0.5 df", Bahia, Brazil) with a sieve size <1.0 mm. The seeds obtained were collected, placed under shaded conditions, and air dried. The dried pitaya seeds were kept in an amber bottle and stored at -20 °C until further use.

2.3. Oil extraction

The pitaya seeds were crushed into small particles using a mortar and pestle. For solvent extraction, 5 g of pitaya seeds were placed into a cellulose paper cone, and oil extraction was performed using light petroleum ether (b.p. 40–60 °C) in a Soxhlet extractor for 8 h [\(AOCS, 1993\)](#page-4-0). The oil was then recovered by evaporating off the solvent using a rotary evaporator (Laborata 4000; Heidolph, Germany), and the residual solvent was removed by flushing with 99.9% nitrogen.

2.4. Fatty acid composition and thermal properties

The fatty acid composition was determined by conversion of the oil to fatty acid methyl esters (FAME) by dissolved 50 mg of oil with 950 μ l of n-hexane followed by the addition of 50 μ l of sodium methoxide using the method of [Cocks and Van Rede](#page-4-0) [\(1966\).](#page-4-0) The mixtures were vortexed for 10 s and allowed to settle for 5 min. The top layer $(1 \mu l)$ was injected into an Agilent 6890N (Little Falls, DE, USA) gas chromatograph equipped with a split– splitless injector and a flame ionisation detector. A polar capillary column BPX70 (0.32 mm internal diameter, 30 m length, and 0.25 µm film thickness; SGE International Pty. Ltd., Victoria, Australia) was used at a column head pressure of 10 psi. Helium (99.995%) at approximately 23 ml/min (measured at an oven temperature of 150 \degree C) was used as the carrier gas, and nitrogen (99.999%) at 20 ml/min was used as the makeup gas. The flame ionisation detector (FID) and injector temperatures were both maintained at 220 °C. The initial column oven temperature was 115 °C, and the temperature was programmed to 180 °C at 8 °C/ min and held there until the analysis was completed. The FAME peaks were identified by comparison with the retention times of a standard mixture. The peak areas were computed, and the percentages of the FAME were obtained as area percentages by direct normalisation. Iodine value (IV) analysis according to the AOCS Official Method was employed for the seed oil samples. All determinations were done in triplicate.

The thermal properties were analysed by DSC ([Tan & Che Man,](#page-4-0) [1999a, 1999b](#page-4-0)). For the DSC analysis, a PerkinElmer Diamond DSC differential scanning calorimeter, equipped with Pyris data analysis software (PerkinElmer Corp., Norwalk, CT), was used. Nitrogen (99.999% purity) was the purge gas and flowed at \sim 20 ml/min. Samples of 3–5 mg were weighed into aluminium pans to the nearest 0.1 mg, and covers were hermetically sealed into place. An empty, hermetically sealed aluminium pan was used as a reference. Prior to the analysis of the samples, the baseline was obtained with an empty, hermetically sealed aluminium pan.

2.5. Chromatographic analysis of α , β , γ , δ -tocopherols

The tocopherol content was determined according to a modified method of [Gonzalez, Pablos, Martin, Leon, and Valdenebro \(2001\).](#page-4-0) The tocopherols were analysed by an HPLC system (Shimadzu, Kyoto, Japan). The chromatographic system included a Shimadzu Fluorescence detector and a 250×4 mm LichroCART Purospher STAR Si 5 um column (Merck, Darmstadt, Germany). The separation of all of the tocopherols was based on mobile-phase hexane/ propan-2-ol (99:1, v/v) at a flow rate of 1.0 ml/min with detection at 290 nm. One gram of seed oil was diluted with 10 ml of hexane, the aliquots of this solution were filtered $(0.45 \mu m)$ nylon syringe filter), and 20 μ l samples were injected for the HPLC analysis.

2.6. Phenolic acid

2.6.1. Phenolic acids extraction

The phenolic acids were extracted based on a modified method of [Mateos et al. \(2001\)](#page-4-0). A diol-bonded phase cartridge was conditioned by the consecutive passing of 6 ml of methanol and 6 ml of hexane. The filtered oil sample (2.5 g) was weighed. The oil was dissolved in 6 ml of hexane and applied to the column; the solvent was pulled through, whilst the sample was left in the solid phase. The sample container was washed with two 3-ml portions of hexane, which were run out of the cartridge. The sample container was washed again with 4 ml of hexane/ethyl acetate $(90:10, v/v)$, which was run out of the cartridge and discarded. Finally, the column was eluted with 10 ml of methanol, and the solvent was evaporated in a rotary evaporator at 35° C under a vacuum until dried. The residue was extracted with $500 \mu l$ of methanol/water (1:1, v/v) at 40 °C. An aliquot (20 µl) of the final colourless solution was injected into the HPLC system.

2.6.2. Chromatographic analysis of phenolic acids

The chromatographic analysis was performed in a JASCO liquid chromatographic system MD 2010 Plus equipped with a diode array UV, PDA detector (Jasco Corporation, Kyoto, Japan). A LiChrosorb RP-18 column $(10 \mu m)$ (Merck, Darmstadt, Germany) at room temperature was used. The elution was performed at a flow rate of 1.0 ml/min, using a mobile phase consisting of 1.5% (v/v) acetic acid in water-methanol 82:18 (v/v). The chromatograms were acquired at 280 nm. The identification of the phenolic acids was based on the retention times in comparison to the standards. The quantification of the phenolic acid was carried out using an external standard method. A stock solution of the standard was prepared at a concentration of 1 mg/ml of each compound diluted in methanol. The standards solutions were injected into the HPLC system at various concentrations (0.02, 0.04, 0.06, 0.08, and 0.10 mg/ml). The calibration curves ($R^2 \ge 0.99$) were constructed with the external standard method, correlating the area of the peaks with the concentration.

2.7. Gas chromatography analysis of sterols

2.7.1. Saponification for sterol analysis

The separation of the sterols was performed after the saponification of the oil sample according to the modified method of [Ram](#page-4-0)[adan, Shahwan, and El-Sanhoty \(2006\)](#page-4-0). The total lipid (250 mg) and 100 μ l of 5 α -cholestane (1 mg/ml) were refluxed with 5 ml of ethanolic potassium hydroxide solution (6%, w/v) for 60 min. The unsaponifiables were first extracted three times with 10 ml of petroleum ether, the extracts were combined and washed three times with 10 ml of neutral ethanol–water $(1:1, v/v)$, and then they were dried overnight with anhydrous sodium sulphate. The extract was evaporated at 25 \degree C under reduced pressure. The residual solvent was removed by flushing with nitrogen (99.9%).

2.7.2. Preparation of trimethylsilyl ether (TMS) derivatives of sterols

 $N,$ O-bis(trimethylsilyl)trifluoroacetamide (BSTFA) (125 μ l) with 1% trimethylchlorosilane (TMCS) was added to the dry residue, and then the mixture was vortexed for about 10 s and heated at 70 \degree C for 30–60 min. After cooling, $1 \mu l$ aliquots were directly injected into the gas chromatograph.

2.7.3. Gas chromatography–flame ionisation detection (GC–FID)

GC–FID analyses were performed using the analytical conditions reported by [Cunha, Fernandes, and Oliveira \(2006\)](#page-4-0). Briefly, a gas chromatograph, Agilent 6890N (Little Falls, DE, USA), equipped with a split–splitless injector, an FID, and an MDN-5 – Supelco (30 m \times 0.32 mm i.d., 0.25 µm film thickness) column was used. The initial column temperature was 250° C and programmed to increase at a rate of $2^{\circ}C/m$ in to 300 $^{\circ}C$, and it then remained constant for 12 min.

2.8. Statistical analysis

In this study, a Student's t-test was used to differentiate between the two different species of seed oils. All numerical data are expressed as the arithmetic average ± standard deviation from the three replicates. The analyses were carried out using MINITAB 13 (Minitab Inc., Pennsylvania, USA). The significance value for all of the analyses was defined at $P < 0.05$.

3. Results and discussion

Table 1 shows the two Hylocereus cacti species characteristics and the seed oil content. The percentages of oil that was extracted from the H. polyrhizus seed oil, RFSO (red flesh seed oil), and the H. undatus seed oil, WFSO (white flesh seed oil), were 18.33% and 28.37%, respectively. The oil content of the H. undatus seed (white flesh) was significantly ($P < 0.05$) higher than that of the H. polyrhizus seed (red flesh). Surprisingly, the oil contents of the RFSO and WFSO obtained in this study were lower than those (29.50% and 32.0%) reported by [Ariffin et al. \(2009\)](#page-4-0). The percentage yields of seeds from mature pitaya fruit were 14.27% from the H. polyrhizus red flesh and 14.67% from the H. undatus white flesh. The total yield of seeds from both pitaya species obtained in this study compared to the 1.3–1.5% that was reported by [Ariffin et al.](#page-4-0) [\(2009\)](#page-4-0) showed that a higher percentage of seed was collected by using the sieving machine. The iodine value (IV) analysis was carried out to measure the degree of unsaturation in the seed oil samples. From Table 1, the IV for the WFSO was significantly $(P < 0.05)$ higher than the RFSO. The high value of the IV indicates that WFSO (101.23) contains a higher degree of unsaturation compared with RFSO (88.43).

The principal fatty acid components in both pitaya seed oils were palmitic (C16:0), oleic (C18:1), and linoleic (C18:2) acids (Table 2). The total unsaturation of the fatty acids for pitaya seed oil, 77.22–82.01%, was comparable to the value for soybean oil, 84.6% ([Tarandjiiska, Marekov, Nikolova-Damyanova, & Amidzhin, 1996\)](#page-5-0), and grape seed oil, 59–79% [\(Wang, Liao, & Zhang, 1996](#page-5-0)). It was observed that in both WFSO and RFSO, polyunsaturated fatty acids (PUFAs) were higher than monounsaturated fatty acids (MUFAs). Linoleic acid was the main fatty acid for both species of seed oil (RFSO 48.00%, WFSO 55.63%), followed by oleic acid (RFSO

Table 1

Characteristic of pitaya fruits and seed oil.

 a Values are means of triplicate determinations \pm SD. Means within each row with different letters (a, b) are significantly ($P < 0.05$) different.

 b Percentage of oil = (weight of extracted oil/weight of seed) \times 100.

Table 2

Fatty acid compositions of pitaya seed oil (g/100 g oil).

 a Values are means of six determinations from two replicate experiments \pm SD. Means within each row with different letters (a, b) are significantly $(P < 0.05)$ different.

26.80%, WFSO 24.43%). [Ariffin et al. \(2009\)](#page-4-0) obtained similar results in H. polyrhizus seed oil (linoleic 49.6%, oleic 21.6%) and H. undatus seed oil (linoleic 50.1%, oleic acid 23.8%). Their measured percentage of oleic acid is lower than our result; there are two fatty acids, arachidic acid (C20:0) and erucic acid (C22:1), that have been identified in this study, but were not reported by [Ariffin et al. \(2009\).](#page-4-0) Linoleic acid derivatives serve as structural components of the plasma membrane and as precursors of some metabolic regulatory compounds. The results show that in both species of pitaya seed oil, about 18.00–22.78% of the fatty acids present are saturated, 25.20–27.92% are monounsaturated, and 49.30–56.81% are polyunsaturated. Thus, it seems that pitaya seed oil contains a substantial amount of essential fatty acids.

The thermal curves for the RFSO and WFSO at different heating and cooling rates are given in [Figs. 1 and 2,](#page-3-0) respectively. Their corresponding transition temperatures are given in [Table 3](#page-3-0). The crystallisation curves for the WFSO and RFSO samples crystallised at a series of cooling rates are illustrated in [Fig. 2](#page-3-0). From [Table 3](#page-3-0), both of the crystallisation curves have two exothermic peaks. In the cooling profile, the WFSO crystallised at -35.95 °C and -6.41 °C, whilst the RFSO crystallised at -36.22 °C and -5.28 °C. In the DSC melting curves from the heating rate studies, the melting curve for RFSO consisted of four peaks, whilst the WFSO curve consisted of five peaks [\(Fig. 1\)](#page-3-0). In the WFSO and RFSO samples, one major melting peak was exhibited as a single, tall endotherm peak shown at b_2 (–25.76 °C) and a₂ (–25.39 °C), respectively. Differential scanning calorimetry (DSC) can use in detecting or verifying adulteration. In the oil industry, the DSC cooling/melting thermograms give valuable information on the thermal properties of fat products ([Biliaderis, 1983\)](#page-4-0). Every oil has own unique characteristic of fatty acids and triacylglycerol (TAG) profiles. Five endothermic peaks were found in WFSO may be due the presence of different amount of TAG compounds as compared to RFSO (only four endothermic peaks). The present of small shoulder endothermic peak [\(Fig. 1,](#page-3-0) curve B, peak b4) may be ascribed to the melting of TAG with a combination of unsaturated and saturated fatty acids in the glycerol moiety, as previously reported ([Tan & Che Man, 2000\)](#page-5-0).

Two types of seeds, RFSO and WFSO, were examined, and α tocopherol was the major component, constituting 31.90 mg/ 100 g (\sim 73%) and 24.00 mg/100 g (\sim 65%), respectively [\(Table 4\)](#page-4-0). Meanwhile, γ -tocopherol accounted for 11.60 mg/100 g of RFSO and 12.70 mg/100 g for WFSO. RFSO contained a statistically (P < 0.05) higher amount of α -tocopherol than WFSO. α -Tocopherol is the major tocopherol in olive oil $(\sim 93\%)$, sunflower oil $(\sim 96%)$, and in prickly pear peel oil (Opuntia ficus indica L.)

Fig. 1. Differential scanning calorimetry melting curves of RFSO (A) and WFSO (B). Refer to Table 3 for transition temperatures.

Fig. 2. Differential scanning calorimetry crystallisation curves of RFSO (A) and WFSO (B). Refer to Table 3 for transition temperatures.

(\sim 80%), whilst γ -tocopherol is predominant in cactus pear seed oil $(\sim80\%)$, soybean oil ($\sim63\%$), and canola oil ($\sim63\%$) ([Degreyt, 1998\)](#page-4-0). In general, α -tocopherol and γ -tocopherol comprise more than 60% of the total vitamin E content in a majority of vegetable oils, with some exceptions, as in palm oil, which is characterised by a high amount of α -tocopherol (\sim 30%) and tocotrienols (\sim 60%) [\(Degreyt,](#page-4-0) [1998\)](#page-4-0). β -Tocopherol and δ -tocopherol were not detected in RFSO and WFSO.

[Table 4](#page-4-0) shows that the seven phenolic compounds that were identified in RFSO were protocatechuic acid (0.93 mg/100 g), the highest relative content, followed by p-coumaric (0.78 mg/100 g), p-hydroxybenzoic (0.66 mg/100 g), vanillic acid (0.64 mg/100 g), gallic acid $(0.25 \text{ mg}/100 \text{ g})$, caffeic $(0.08 \text{ mg}/100 \text{ g})$, and syringic acid (0.08 mg/100 g). However, for WFSO, protocatechuic

Table 3

Comparison of differential scanning calorimetry transition temperatures for melting and crystallisation curves of pitaya seed oil samples.

Curve	Sample	Transition temperature $(°C)$				
Melting ^a	RFSO (A) WFSO(B)	-37.04	-25.76 -35.73 -25.39 -16.77	-12.90	7.40 -5.64	5.46
Crystallisation ^a	RFSO(A) WFSO(B)	-36.22 -35.95	-5.28 -6.41			

Abbreviations: RFSO, H. polyrhizus seed oil (red flesh); WFSO, H. undatus seed oil, (white flesh).

^a Values are means of triplicate determinations \pm SD. See Figs. 1 and 2 for thermal curves.

Table 4

Tocopherol, phenolic acid and phytosterol compositions of pitaya seed oils.

nd – Not detected.

^a Values are means of six determinations from two replicate experiments \pm SD. Means within each row with different letters (a, b) are significantly (P < 0.05) different.

(0.93 mg/100 g) was present in the highest amount, followed by p coumaric (0.79 mg/100 g), p-hydroxybenzoic (0.72 mg/100 g), caffeic $(0.71 \text{ mg}/100 \text{ g})$, vanillic acid $(0.70 \text{ mg}/100 \text{ g})$, syringic $(0.21 \text{ mg}/100 \text{ g})$, and gallic acid $(0.20 \text{ mg}/100 \text{ g})$.

Sterols are important constituents of oils because they relate to their quality and are widely used to check authenticity (Salvador, Aranda, Gomez-alonso, & Fregapane, 2001). Phytosterols are of interest due to their antioxidant activity and impact on health. This fraction has been considered as the major unsaponifiable fraction in many oils. The sterol composition in the pitaya seed oils are presented in Table 4. From the data, both the RFSO and WFSO contain an abundance of β -sitosterol, 676 mg/100 g and 548 mg/100 g, respectively. High β -sitosterol content was also found in majority of vegetable oils, such as olive oil, grape seed oil, groundnut oil, sunflower oil and date seed oil, in which the mean relative contents were 84.3%, 69.2%, 62.3%, 61.9% and 80.98% of total sterols, respectively (Feinberg, Favier, & Ireland-ripert, 1987). RFSO also contained 252 mg/100 g campesterol, whilst WFSO contained 198 mg/100 g. Stigmasterol was found in the RFSO at a level of 106 mg/100 g, which was significantly higher than the level of 72 mg/100 g that is found in the WFSO. The data showed no remarkable differences between RFSO and WFSO in terms of the sterol content and composition. Many studies have shown that high levels of total low density lipoprotein cholesterol (LDL-cholesterol) increase risk factors, whereas a high concentration of high density lipoprotein cholesterol (HDL-cholesterol) or a low LDL/ HDL ratio may protect against coronary heart disease ([Tapiero,](#page-5-0) [Townsend, & Tew, 2003\)](#page-5-0). The sterols from vegetable oils have been shown to lower the total and LDL cholesterol levels in humans by inhibiting cholesterol absorption from the intestine ([Tapiero et al.,](#page-5-0) [2003\)](#page-5-0).

4. Conclusions

The results showed that H. polyrhizus seed oil (RFSO) differed from H. undatus seed oil (WFSO) by showing a lower oil content, fatty acid composition, phenolic compounds, and a higher tocopherol content. These differences are probably influenced by the different natures of the species of Hylocereus cacti. Both pitaya species contained a high amount of linoleic acid as compared with grape seed, flaxseed, and rapeseed (canola) oils. It is evident that pitaya seeds provide a high yield of oil (18.33–28.37%) and are able to serve as a potential source of natural antioxidants such as phenols, tocopherols, and sterols. A waste product, such as pitaya seeds from the juice industry, could serve as a source of edible oil. Thus, this study consolidates the possibility of incorporating pitaya seed oil into cosmetics, pharmaceuticals, and food.

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