

INTER-VARIETAL VARIATION IN THE COMPOSITION OF SEEDS AND SEED OILS FROM WINTER MELON [*BENINCASA HISPIDA* (THUNB.) COGN.] FRUIT

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Abstract

Winter melon (*Benincasa hispida*), locally known as Kundur, is a vegetable crop, popular, especially among Asian communities both for nutritional and medicinal attributes. In the present work, physicochemical properties of seeds and the extracted seed oils were examined and compared among three cultivars namely round, oval and hybrid of winter melon. The seeds from round, oval and hybrid fruits, exhibited protein, fiber and ash contents 28.18-42.03, 19.36-26.21 and 5.02-11.81%, respectively. The oils were extracted based on Soxhlet method by petroleum ether, whilst yields ranged from 17.78-32.53% (wt/wt). The extracted oils were analyzed for physicochemical parameters, and fatty acids, tocopherols and sterols profiles. The results for specific gravity (25°C), refractive index (n_D 25°C), iodine value (IV), saponification value (SV), peroxide value (PV), and free fatty acid (% as oleic acid) were 0.89-0.91 g/mL, 1.4627-1.4646, 119.9-125.1 g I/100 g oil, 182.3-194.1 mg KOH/ g oil, 1.13-1.33 mequiv.O₂/kg, 1.57-2.10%, respectively. The oil color intensity in terms of yellow and red units was 6.9Y + 1.0 R to 8.9Y + 1.9R. The amounts of oil tocopherols as analyzed by HPLC varied widely among the cultivars tested showing α -tocopherol 31.1-207.6 mg/kg and δ -tocopherol 60.4-146.0 mg/kg. According to the GLC analysis linoleic acid (C18:2) was established to be the principal fatty acid (63.10-70.64%) followed by C16:0 (12.45-17.59), C18:1 (8.46-12.87%) and C18:0 (5.13-7.48%). Analysis of oil sterol fractions, using GC and GC-MS, revealed the presence of β -sitosterol (54.62–60.50%), campesterol (15.10–18.50%), stigmasterol (11.00–14.30% and Δ^5 -avenasterol (6.40–8.14 %) as the four main components. Most of the properties of the seed oils analyzed varied significantly among fruit cultivars tested. Overall, we concluded that the seeds, which are under-utilized and often discarded as an agrowaste, from winter melon should be explored for extraction of high-linoleic oil with additional tocopherols and phytosterol benefits.

Introduction

Winter melon [*Benincasa hispida* (Thunb.) Cogn.] (synonym; *Benincasa cerifera* Savi), a member of the family Cucurbitaceae, is known as one of the important vegetable crops that are popular not only due to nutritional but also for medicinal uses (Grubben, 2004; Yadav *et al.*, 2005, Zaini *et al.*, 2011). Commonly known with several English names such as white gourd, winter melon, ash gourd, ash pumpkin, Chinese wax gourd, wax gourd and gourd melon, the plant has different local names, for example *Bleego* (Indonesian), *Dong Gua* (Chinese and Korean), *Petha* (Hindi), *Tougan* (Japanese), *Bí dao* (Vietnamese), *Fak kio* (Thailand), *Kondol* (Philippines) and *Kundur* (Malay) *etc.*, (Morton, 1971; Robinson & Decker-Walters, 1999; Marr *et al.*, 2007). According to Walters & Decker-Walters (1989) and Bates & Robinson (1995), there are four recognized cultivars of winter melon fruit viz., unridged winter melon, ridged winter melon, fuzzy gourd and wax gourd mainly characterized based on their shape, size, fuzziness, waxiness, and presence or absence of a dusty or ashy layer. However, Marr *et al.*, (2007) reported 16 cultivars of winter melon based on the fruit shape, length, and width as well as skin color. In Malaysia, winter melon, with local name “*Kundur*” is mainly represented by two cultivars namely round and oval, however a hybrid-type round (developed through breeding of the green winter melon genotype and fuzzy white gourd genotype) is also grown (Zaini *et al.*, 2011). *Kundur* fruit in Malaysia, although cultivated on a considerable area, is underutilized. Johor, Pahang, Perak Sabah, Kelanran and Selangor are some of the important states cultivating this fruit species mainly for vegetable purposes.

Almost all parts (leaves, flower, fruit and seed) of *Benincasa hispida* (*B.hispida*) have been used, either as

food or as medicine. The young shoots, leaves and flowers can be used as vegetable. The immature as well as mature, large size fruits are often cooked as vegetable, stuffed and steamed or chopped into small blocks candied with sugar. In China, India, Nepal, Cuba and Southeast Asian regions, the mature fruits are used in preparation of soups, while these are also sliced and eaten as cooked alone or with meat and as well as incorporated in the preparations of other dishes (Wu, 1987; Rahman *et al.*, 2006; Marr *et al.*, 2007).

B. hispida fruit is a good source of valuable nutrients including organic acids, natural sugars, amino acids, vitamins and mineral elements (Wills, 1984; Mingyu *et al.*, 1995; Zaini *et al.*, 2011). A number of biological and medicinal properties such as anti-obesity (Kumar & Vimalavathini, 2004), anti-inflammatory (Huang *et al.*, 2004), anti-diarrhoeal (Mathad *et al.*, 2005), anti-pyretic (Qadrie *et al.*, 2009), anti-compulsive (Girdhar *et al.*, 2010), antioxidant (Gill *et al.*, 2010), anti-ulcer and diuretic (Grover *et al.*, 2001; Rachchh & Jain, 2008) have been ascribed to *B.hispida* (Zaini *et al.*, 2011). As a potential source of wide array of functional bioactives and therapeutics viz., phenolics, triterpenes, glycosides and sterols, the fruit has been widely used for the treatment of ulcer, epilepsy, diabetic complications, hypertension, nervous disorders and Alzheimer disease in the traditional medicine system of Asian communities (Mingyu *et al.*, 1995; Yoshizumi *et al.*, 1998; Choi *et al.*, 2001; Kumar & Vimalavathini, 2004; Lee *et al.*, 2005, Moon *et al.*, 2008; Gill *et al.*, 2010). A comprehensive review article highlighting the nutritional, medicinal and pharmacological properties of this multipurpose fruit has recently been published (Zaini *et al.*, 2011).

A native of Southeast Asia, *B.hispida* is now widely grown in East Asia and South Asia. Normally found in the form of wide-spreading, hairy, monocious vine with branched tendrils reaching a length of 4-8 meters, the plant bears large green fruits which have wide variability

among its cultivars. The fruits, globose-cylindrical in shape, 25–40 cm long, and covered with thick epicuticular wax, can be stored for a longer period *ca.* one to two years if the waxy surface is not cleaned off, otherwise the fruit would quickly spoil and deteriorate (Morton, 1971; Rahman *et al.*, 2006; Marr *et al.*, 2007; Pandey *et al.*, 2008). The yields of young fruits, often harvested 60–100 after sowing, is more than 30 t/ha, while in India, typically mature wax gourd yields is about 20 t/ha. A seed yield between 100 and 150 kg/ha is recorded from India and 200–300 kg/ha from Thailand (Grubben, 2004). The flesh is white to dull green, with a specific bland flavor. The seeds are many, oblong, smooth, and may range in length from 1.0 to 1.5 cm while in width 0.5 to 0.8, depending on the cultivar and shape of fruit. The seeds, packed in the center of the fruit, change their color from white to yellowish brown as the fruit matures (Morton, 1971; Raveendra *et al.*, 2006). The seeds, which also have considerable amount of oil, can be fried and used as a snack. According to Martin (1984) *B. hispida* fruit seed is perhaps one of the best potential sources of cucurbit seed oils for the hot and humid tropics. The oil from *B. hispida* seeds is reported to be good for the brain and also beneficial in curing syphilis (Qadrie *et al.*, 2009). Our preliminary investigation (Sew *et al.*, 2010) revealed that the oil contained high proportion of linoleic acid (C18:2 ω -6), accounting for more than 65% of the total fatty acids. Oleic and palmitic were the other two important fatty acids detected in the oil.

Currently, the uses of winter melon (*B. hispida*) fruit are mostly due to its food and medicinal benefits, rather than due to production of byproducts such as seed oil and waxes or other value-added products. As winter melon fruits produce large amount of seeds, often discarded as agrowaste, therefore the seeds can be explored as a potential source of oil for value-addition. Regardless of the high nutritional and medicinal potential, availability and cultivar diversity in plant and fruit characters, no efforts have yet been made on studying full physicochemical characteristics of seed oils from different cultivars of winter melon fruit. The present study was undertaken with the main objective to evaluate the detailed physicochemical composition of the oils extracted from seeds of three locally cultivated cultivars viz., oval, round and hybrid of Malaysian winter melon. The quality-oriented routine physicochemical attributes together with profile of fatty acids, tocopherols and sterols of the seed oils extracted were analyzed and compared among cultivars tested.

Materials and Methods

Seeds: Oval winter melon (*B. hispida*) fruit was purchased from a local market in Serdang, Selangor, Malaysia whereas round and hybrid (round) were harvested from winter melon fruit farms in Sungai Lang, Sabak Bernam, Selangor, Malaysia and Temerloh, Pahang, Malaysia, respectively. Three different fruit samples were assayed for each cultivar and the seeds were separated from the fruits manually after cutting with a sharp steel knife. The seeds were cleaned and oven-dried at 40°C for 24 h. The seeds were ground using a coffee grinder; the material with uniform size, passing through 80-mesh sieve was used for extraction purposes.

Oil extraction: The ground seed materials were extracted with 200 mL petroleum ether using Soxhlet apparatus for

6 h on water bath according to AOAC Official Method, 948.22 (Anon., 2000). After extraction, the excess of the solvent was removed under reduced pressure using a rotary vacuum evaporator (Eyela Co. Ltd., Tokyo, Japan). The recovered oil was freed of any traces of moisture by mixing with small amount of anhydrous Sodium sulphate and then filtered. The oil was capped in a dark brown sample vial and stored below 5°C until used for further analyses.

Proximate analysis: The oil seed residues were analyzed for crude fiber, ash and protein content according to the AOAC Official Methods 935.53, 930.05, 920.152, respectively (Anon., 2000). About 3 to 5 g of the sample was taken for crude fiber and ash analyses. Percent (%) of crude fiber was equal to the weight of residue without ash divided by the weight of sample, then multiplied by 100. The ash content was determined by incinerating the sample in muffle furnace at 500°C for 6 h. For determination of protein content, the tested sample was digested using copper catalyst and sulphuric acid, distilled and then titrated by 0.05N H₂SO₄ for nitrogen determination. The amount of protein was calculated by multiplying % of nitrogen determined by the conversion factor 6.25 according to Kjeldhal method (Anon., 2000).

Physicochemical properties of oils: The physicochemical properties including specific gravity (25 °C), iodine value, saponification value, peroxide value, and free fatty acids of the extracted seed oils were determined in accordance with AOAC Official Methods; 920.212, 993.20, 920.160, 965.33, and 940.28, respectively (Anon., 2000). The refractive index was measured using Refractometer (Bellingham + Stanley Limited RFM 110) at 25°C and while the color intensity was read by Lovibond Tintometer (Tintometer Ltd., Salisbury, Wiltshire, United Kingdom).

Tocopherol analysis: Quantification of tocopherols was carried out according to the method described by Arranz *et al.*, (2008) with minor modifications. Water HPLC system equipped with Waters 2487 Dual Wavelength Absorbance Detector, Waters 600 Pump was used. Briefly, 100 mg oil was dissolved in 1 mL hexane and filtered through 0.45 μ m nylon membrane filter. A 20- μ L sample volume was injected onto ACA 5Sil column (250 x 4.6 mm) at room temperature (28 \pm 1°C) using the mobile phase flow rate of 1.0 mL/min. The mobile phase used was a mixture of hexane and 2-propanol (98:2, v/v). Tocopherol separation was operated by isocratic elution of the mobile phase and was controlled by Water Empower2 software (Waters, Milford, MA). The unknown peaks, detected at a wavelength of 295 nm, were identified by matching their retention time with those of pure standards of α -, and δ -tocopherols. For quantification, peak area-based standard calibration curve, constructed by analyzing tocopherols standard solutions over concentration range of 10 to 200 mg/L was employed. The concentration of tocopherols was reported as mg/kg of oil.

Fatty acid methyl esters (FAMES) preparation and gas chromatographic (GC) analysis: FAMES were prepared following a standard procedure as described by Christie (1993). A 50- μ L oil sample, dissolved in 950 μ L hexane, was transmethylated using Sodium methoxide. The analysis of FAMES was performed by GC (Hewlett-Packard 6890; Agilent, Wilmington, DE, USA) fitted with

a flame ionization detector (FID). A fused silica capillary BPX-70 column (30 m x 0.32 mm, 0.25 µm film thickness) was used for separation. The initial column oven temperature was set at 115°C and programmed to 180°C @ 8°C/min. The temperature was further raised to 240°C @ 8°C/min and held for 10 min. The sample volume injected on to the capillary column was 1 µL using splitless injection mode. Helium was used as a carrier gas at a flow rate of 1.6 mL/min. The unknown FAMES were identified by comparing their retention times with those of pure standards. The fatty acids composition was expressed as relative percentage of the total peak area.

Phytosterol analysis: The oils phytosterols were analyzed following a previously described method (Rudzin'ska *et al.* 2003; Anwar *et al.*, 2008). Briefly, 500 µg of 5- α -cholestane dissolved in chloroform was added to 0.5 g oil as an internal standard. The oil mixture was then subjected to saponification using 1 M methanolic KOH at room temperature for 18 h. The unsaponifiable matter was extracted with diethyl ether and the sterols fractions were silylated with Sylon BTZ (Sigma). The derivatives of sterols prepared were analyzed on a Perkin-Elmer gas chromatograph model 8700 coupled with a HP-5 capillary column (30 m x 0.25 mm, film thickness 0.25 µm; Agilent Technologies, Little Falls, CA, USA) and a flame ionization detector (FID). Separation was done isothermally at 285°C, while the injector and FID temperatures were set at 300°C. Extra-pure helium was used as a carrier gas at a flow rate of 1.5 mL/min. Unknown sterol components were identified on the basis of matching their retention times with those of pure standards. The sterol composition was expressed as relative percentage of the total peak area. The samples were injected in a split mode (1:50). Further identification and authentication of phytosterols was done by GC-MS using an Agilent-Technologies 6890N Network gas chromatographic (GC) system, equipped with Agilent-Technologies 5975 inert XL Mass selective detector and Agilent-Technologies 7683B series auto injector (Agilent-Technologies, Little Falls, CA, USA, under the same chromatographic conditions as described above.

Statistical analysis: All experiments were conducted in triplicate and the statistical significance differences of mean were calculated using SAS (8.1), with the help of one-way ANOVA; results are expressed as means \pm SD. A probability value at $p < 0.05$ was considered to denote the statistically significant differences.

Result and Discussion

Proximate composition of seeds: In the present study physicochemical properties of seeds and the extracted seeds oils from fruits of three cultivars viz., cultivar 1

(round shaped), cultivar 2 (oval shaped) and cultivar 3 (hybrid round shaped) of Malaysian winter melon were appraised and compared. The contents of oil, protein, fibre and ash ranging from 17.78% to 32.53%, 19.36% to 26.21%, 28.18% to 42.03%, and 5.02% to 11.81%, respectively, varied significantly ($p < 0.050$ among the cultivars tested (Table 1). Cultivar 3 had the highest oil yield and fiber content followed by Cultivar 2 and Cultivar 1. On the other hand, the amount of protein and ash were higher in Cultivar 1 as compared to Cultivar 2 and Cultivar 3. The present work showed somewhat different data to those reported by Sew *et al.*, (2010) who analyzed the seeds of only hybrid (round) winter melon cultivar and found 20.70% of oil, 11.63% of protein, 45.00% of crude fiber, and 4.10% of ash. Presently observed variations in proximate parameters of seeds might be attributed to the varying harvesting regime and origin of the fruit used in these studies. Interestingly, like other cucurbit seeds, winter melon seeds in the present analysis were found to be a good source of food protein and oil.

Winter melon seeds in the present study expressed a higher range of protein content among the other species of cucurbits whereas a comparable amount of fiber and acceptable ash content. Mariod *et al.*, (2009) investigated nutritional composition of *Citrullus lanatus*, *Cucumis prophetarum*, *Cucumis sativus*, *Luffa echinata*, and *Cucumis melo* seed from Sudan and found values for protein, fiber, ash in the range of 14.50-17.50, 25.86-36.04, and 3.41-8.33%, respectively. Cucurbits seeds from different species have been reported as an impressive source of oil with yields varying between 26.9% and 56.67%. However, the seeds of cucurbit species: *Citrullus lanatus*, *Cucumis prophetarum*, *Cucumis sativus*, *Luffa echinata*, and *Cucumis melo* from Sudan contained oil in the range of 10.9-27.10% (Mariod *et al.*, 2009). In another study, the seeds from *Cucumeropsis mannii*, *Cucurbita maxima*, *Cucurbita moschata*, *Lagenaria siceraria* and *Cucumis sativus*, cultivated in Cameroon, showed a much higher oil content ranging from 40% to 56% (Fokou *et al.*, 2009). Furthermore, water melon, wild melon, hybrid melon, bitter melon, egusi melon, gourd, pumpkin, cucumber, loofah seeds cultivated in Turkey, Bangladesh, Ivory Coast, Egypt, Nigeria, Iran, Serbia and Malaysia exhibited 52.00% (Paksoy *et al.*, 2010), 33.93-36.21% (Ali *et al.*, 2008), 42.67-56.67% (Loukou *et al.*, 2007), 50.10-51.01% (El-Adawy & Taha, 2001), 13.15-56.5% (Badifu, 1991, Dawodu, 2009, Anhwange *et al.*, 2010), 50.00% (Baboli & Safe Kordi, 2010), 22.1% (Milovanovic & Picuric-Jovanovic, 2005), 29.8-33.55 (Ismail *et al.*, 2010) oil yields, respectively.

Table 1. Proximate composition of winter melon (*B. hispida*) fruit seed.

Seeds	Cultivar 1	Cultivar 2	Cultivar 3
Oil content (%)	17.78 \pm 1.31 ^c	24.68 \pm 1.67 ^b	32.53 \pm 0.91 ^a
Protein (%)	26.21 \pm 1.63 ^a	19.36 \pm 0.61 ^b	21.19 \pm 1.63 ^{ab}
Fibre (%)	28.18 \pm 0.9 ^c	32.89 \pm 1.7 ^b	42.03 \pm 2.74 ^a
Ash (%)	11.81 \pm 0.33 ^a	7.48 \pm 0.05 ^b	5.02 \pm 0.02 ^c

Means with different letters within the same row denote significant differences among cultivars ($p < 0.05$); Cultivar 1, round fruit; Cultivar 2, oval fruit; Cultivar 3, hybrid (round) fruit.

Physicochemical characteristics of seed oils: The physicochemical attributes of the seed oils tested are given in Table 2. The tested seed oils displayed values of specific gravity (25°C) and refractive index (n_D 25°C) in the range of 0.89-0.91 g/mL and 1.4627-1.4646, revealing considerable difference among the fruit cultivars (Table 2). The present values were almost comparable with those of other cucurbits such as white melon, gourd, pumpkin, and water melon seed oils from Nigeria, 0.896-0.93 g/mL (Badifu 1991, Yusuf *et al.*, 2006, Essien *et al.*, 2009, Anhwange *et al.*, 2010). According to another investigation, the specific gravity of egusi melon seed oil was 0.9053g/cm³ while 0.883g/cm³ for egusi melon oil methyl esters (Giwa *et al.*, 2010). Minzangi *et al.*, (2011) has summarized that seed oils containing specific gravity

within the range of 0.8800-0.9400 g/cm³ are more suitable for edible purposes whereas those with values 0.8114-1.0714 g/cm³ have more potential for biofuels. The present values for refractive index were found to be within the range of some common vegetable oils such as cotton seed oil, mustard oil, ground nut oil, kapok seed oil and rapeseed oil (Rossell, 1991). The refractive index and specific gravity are two physical parameters which provide useful information about the purity of vegetable oils. As such vegetable oils have certain range for these parameters, and deviation of the data from the set specification may indicate adulteration of oil. Both of these parameters are supportive in the assessment of relative purity and identity of oils and fats (Gull *et al.*, 2011).

Table 2. Physicochemical characteristics of winter melon (*B. hispida*) fruit seed oils

Seed oils	Cultivar 1	Cultivar 2	Cultivar 3
Specific gravity (25°C, g/mL)	0.88 ± 0.07 ^c	0.90 ± 0.04 ^a	0.91 ± 0.03 ^b
Refractive index (n_D 25°C)	1.4627 ± 0.002 ^b	1.4646 ± 0.003 ^b	1.4630 ± 0.002 ^b
Iodine value (g I/100 g oil)	119.9 ± 3.2 ^a	124.4 ± 3.3 ^a	125.1 ± 2.7 ^a
Saponification value (mg KOH/g oil)	194.1 ± 1.2 ^a	191.5 ± 1.7 ^b	182.3 ± 2.5 ^c
Peroxide value (mequiv. O ₂ /kg oil)	1.13 ± 0.15 ^a	1.33 ± 0.10 ^a	1.27 ± 0.12 ^a
Free fatty acid (% of oleic acid)	1.87 ± 0.06 ^a	2.10 ± 0.10 ^b	1.57 ± 0.10 ^c
Color (1-in cell)	6.9Y ± 1.0 ^b	8.9Y ± 1.0 ^b	8.9Y ± 0.5 ^b
	1.0R ± 0.3 ^b	1.4R ± 0.4 ^{ab}	1.9R ± 0.2 ^a

Means with different letters within the same row denote significant differences among cultivars ($p < 0.05$); Cultivar 1, round fruit; Cultivar 2, oval fruit; Cultivar 3, round (hybrid) fruit. Y.: yellow, R.: red.

The range of iodine value, which predicts the degree of unsaturation of oil, was 119.9-125.1 g I/100 g oil, cultivar 3 seed oil had the highest iodine value, followed by cultivar 2 and cultivar 1 seed oils, respectively. Cucurbit seed oils, due to large diversity in cultivar, are expected to exhibit iodine values varying over a broad range. For example, Sudanese wild cucurbit seed oils derived from *Citrullus lanatus*, *Cucumis prophetarum*, *Cucumis sativus*, *Luffa echinata*, and *Cucumis melo* expressed 100-114 gI/ 100g oil of iodine value (Mariod *et al.*, 2009) while seed oils from white melon, squash, cucumber, and gourd exhibited iodine value of 66-125 (Fokou, *et al.*, 2009). Meanwhile, de Melo *et al.*, (2000, 2001) and Yanty *et al.*, (2008) investigated that *Cucumis melo* seed oils had iodine value in the range of 109.6-153.4 gI/ 100g. The saponification value was highest in cultivar 1 (194.1 mg KOH/g oil) whereas lowest in cultivar 3 (182.3 mg KOH/g oil). The oils with higher iodine and saponification value are suitable for soft soap and cosmetics production as well as for edible applications (Akanni *et al.*, 2005).

Peroxide value (1.13-1.33 mequiv. O₂/kg oil) were acceptable among three cultivars of winter melon as recorded in Table 2, indicating good oxidative stability of these oils. Rancidity development is mainly referred to peroxide value (PV) and free fatty acids; high value means the quality of the oil is deteriorated. Fresh seed oils often exhibit PV less than 10 mequiv. O₂/kg oil (Anon., 1999) while 20-40 mequiv. O₂/kg oil means the oil is rancid (Adelaja, 2006). Free fatty acid contents in the present analysis were found 1.57 to 2.10% as oleic acid (Table 2). Cultivar 2 expressed the highest value of free fatty acid, followed by cultivar 1 and cultivar 3. Free fatty acids are the products of hydrolysis, formed either due to chemical or lypolytic hydrolysis. Chemical hydrolysis is mostly due to presence of moisture while the lypolytic is

caused due to presence of natural enzymes, especially, lipases. A higher magnitude of free fatty acids indicates a higher degree of hydrolysis and thus poor oil quality. The present values for peroxide and free fatty acids contents were within the range of seed oils of other species of *Cucurbitaceae*. The seed oil from cultivated cucurbits from Egypt contained 3.21-3.60 of peroxide value (mequiv. O₂/kg oil). Similarly, water melon and pumpkin seed oils were reported to have free fatty acids contents between 1.41-1.48% of oleic acid (El-Adawy & Taha, 2001). On the other hand, the free fatty acids contents of *Citrullus lanatus*, *Cucumis prophetarum*, *Cucumis sativus*, *Luffa echinata*, and *Cucumis melo* var. *flexuosus* and *Cucumis melo* var. *agrestis* seed oils were as high as 2.2, 1.9, 1.3, 4.5, 2.8, and 2.7%, respectively (Mariod *et al.*, 2009).

The colour and odour of the oil is an important feature which determines the consumer's acceptability for oil products. Usually, the commercial oils are yellow in color. The tested seed oils were examined for colour intensity (1-in. cell) in terms of yellow and red using a Lovibond Tintometer. The crude oils exhibited color in the range between light yellow for cultivar 1 (6.9Y + 1.0R) and cultivar 2 (8.9Y + 1.0R) to dark yellow for cultivar 3 (8.9Y + 1.9R). In a previous study, seed oil from another cucurbit member, pumpkin had 15.0Y + 10.9R (Tsaknis *et al.*, 1997). Badifu (1991) and Giwa *et al.* (2010) also examined the colour of white melon, egusi melon, gourd, and pumpkin seed oils from Nigeria using Lovibond Tintometer and reported the result within a range of 3.00 to 28.8Y + 0.2 to 2.7R. It has been revealed that melon seed oils showed pale yellow colour (Essien *et al.*, 2009). The colour development in oils is mainly due to presence of some pigments for example, chlorophyll, and carotenoids etc. which are unintentionally extracted during oil extraction process. Removal of such colouring

plant pigments is an important task from commercial view-point. A process named as bleaching is used to cut and remove the colour of oils and fats during processing (Anwar *et al.*, 2008).

Tocopherol composition: Table 3 represents the tocopherols composition of seed oils tested. The contents of α -tocopherol (31.1-207.6 mg/kg) and δ -tocopherol (60.4-146.0 mg/kg) were found to be varied widely ($p < 0.05$) among the seed oils of three cultivars tested. Cultivar 2 had the highest amount of α -tocopherol while

cultivar 1 had the highest amount of δ -tocopherol. Meanwhile cultivar 3 expressed the lowest value of α -tocopherol while cultivar 2 contained the lowest value of δ -tocopherol. Both α -tocopherol and δ -tocopherol act as antioxidant; however the former isomer has more vitamin E potency while the later exhibits strongest antioxidant activity than either α - or β -isomers (Woodall *et al.*, 1997). Presence of considerably high amount of δ -tocopherol, especially in cultivar 1 and 3, together with reasonable amount of α -tocopherol might be linked with the good oxidative stability of these oils.

Table 3. Tocopherol composition of winter melon (*B. hispida*) fruit seed oils.

Seed oils	Cultivar 1	Cultivar 2	Cultivar 3
α -tocopherol (mg/kg)	171.4 \pm 8.0 ^b	207.6 \pm 9.2 ^a	31.1 \pm 8.8 ^c
δ -tocopherol (mg/kg)	146.0 \pm 8.4 ^a	60.4 \pm 3.0 ^c	115.3 \pm 6.4 ^b

Means with different letters within the same row denote significant differences among cultivars ($p < 0.05$); Cultivar 1, round fruit; Cultivar 2, oval fruit; Cultivar 3, round (hybrid) fruit.

Seed oils from cucurbit fruits are valued as a potential source of tocopherols and other bioactives (Nyam *et al.*, 2009). Nakić *et al.*, (2006) revealed that *Cucurbita pepo* seed oil had 454 to 709 mg/kg of total tocopherols with contribution of α -tocopherol (1.52-2.04%), β -tocopherol (96.02-97.31%), δ -tocopherol (0.86-2.32%). Seed oils from some other cucurbits such as *Citrullus lanatus*, *Cucumis prophetarum*, *Cucumis sativus*, *Luffa echinata* and *Cucumis melo* from Sudan contained 14 to 433 mg/kg of total tocopherols of which γ -tocopherol accounted up to 90% (Mariod *et al.*, 2009). Nyam *et al.*, (2009) investigated that Malaysian *Cucurbits*, bitter melon, Kalahari melon and pumpkin seed oils had high amounts, 806.5 mg/kg to 1350.2 mg/kg of total tocopherols of which α -tocopherol, β -tocopherol, γ -tocopherol and δ -tocopherol were at levels of 151.9-442.2, 0-196.6, 539.3-705.6 and 41.4-172.1 mg/kg, respectively.

The concentration of α -tocopherol in the oil from cultivar 1, 171 mg/kg, was observed to be higher than those reported for palm (89) and soybean (99) oils, while comparable with groundnut oil (178), but lower than cotton seed (338), low-erucic acid rapeseed (202) and maize (282) oils (Rossell, 1991). On the other hand, the level of α -tocopherol detected in cultivar 2 oil (207 mg/kg), although higher than those for palm and soybean oils, was considerably lower than for groundnut, cotton seed, low-erucic acid rapeseed and maize oils (Rossell, 1991). The content of α -tocopherol in cultivar 3 was established to be lower than those of most of the common

vegetable seed oils (Rossell, 1991). On the other hand the level of δ -tocopherol (60-146 mg/kg), determined in the present analysis of winter melon seed oils was higher than those of groundnut (7.6), sunflower (0.6), cotton seed (3.3), and low-erucic acid rapeseed (9) oils, but was lower than that in soybean (421) oil (Rossell, 1991), thus, it would contribute towards good oxidative stability and nutritional quality of these oils. Variations in the composition of tocopherols among different vegetable oils are understandable due to varied genetic make of the varieties, harvesting regime, agroclimatic factors, as well as post-harvest conditions.

Fatty acids composition: Fatty acids composition of winter melon seed oils as analyzed by GC (Figs. 1 and 2) is presented in Table 4. The most abundant fatty acid detected in the tested seed oils was linoleic acid, followed by palmitic acid, oleic acid and stearic acid, respectively (Table 4). Cultivar 3 had the highest amount of linoleic acid (70.64%) while cultivar 1 the lowest (63.10%). The range of palmitic acid, oleic acid and stearic acid were 12.45-17.59, 8.46-12.87, and 5.13-7.48%, respectively. Previously, Sew *et al.*, (2010) also found that winter melon seed oil had linoleic acid (67.37%) as the principal component, followed by palmitic acid (17.11%), oleic acid (10.21%) and stearic acid (4.83%), respectively. Winter melon seed oil could be explored as a potential source of omega 6 dietary supplements.

Table 4. Fatty acid composition (%) of winter melon (*B.hispida*) fruit seed oils.

Fatty acids	Short name	Cultivar 1	Cultivar 2	Cultivar 3
Palmitic acid	C16:0	17.59 \pm 0.36 ^a	13.98 \pm 0.12 ^b	12.45 \pm 0.44 ^c
Stearic acid	C18:0	6.26 \pm 0.36 ^b	7.48 \pm 0.36 ^a	5.13 \pm 0.17 ^c
Oleic acid	C18:1 <i>n</i> -9	12.73 \pm 0.12 ^a	12.87 \pm 0.08 ^a	8.46 \pm 0.20 ^b
Linoleic acid	C18:2 <i>n</i> -6	63.10 \pm 0.57 ^c	64.26 \pm 0.34 ^b	70.64 \pm 0.42 ^a
Arachidic acid	C20:4 <i>n</i> -6	0.32 \pm 0.03 ^a	0.31 \pm 0.01 ^a	ND
Linolenic acid	C18:3 <i>n</i> -3	ND	0.25 \pm 0.04 ^b	0.68 \pm 0.03 ^a
Erucic acid	C22:1	0.39 \pm 0.01 ^c	0.50 \pm 0.02 ^b	0.82 \pm 0.02 ^a
Saturated Fatty acids	SFA	23.86 \pm 0.14 ^a	21.46 \pm 0.31 ^b	17.59 \pm 0.54 ^c
Monounsaturated fatty acids	MUFA	13.12 \pm 0.12 ^a	13.38 \pm 0.08 ^a	9.28 \pm 0.22 ^b
Polyunsaturated fatty acids	PUFA	63.43 \pm 0.55 ^c	64.82 \pm 0.38 ^b	71.32 \pm 0.43 ^a

Means with different letters within the same row denote significant differences among cultivars ($p < 0.05$); Cultivar 1, round fruit; Cultivar 2, oval fruit; Cultivar 3, round (hybrid) fruit; ND; not detected

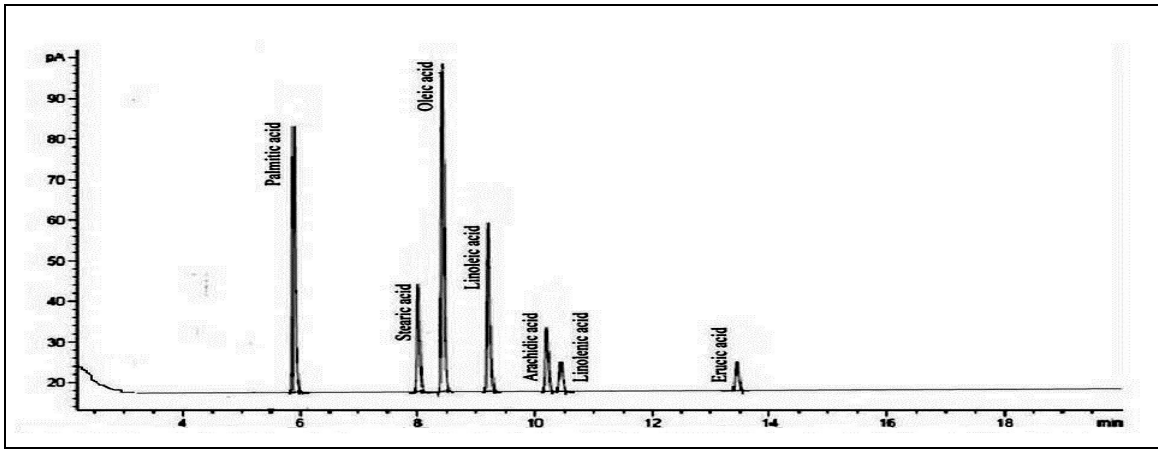


Fig. 1. GC Chromatogram for pure standards of fatty acid methyl esters.

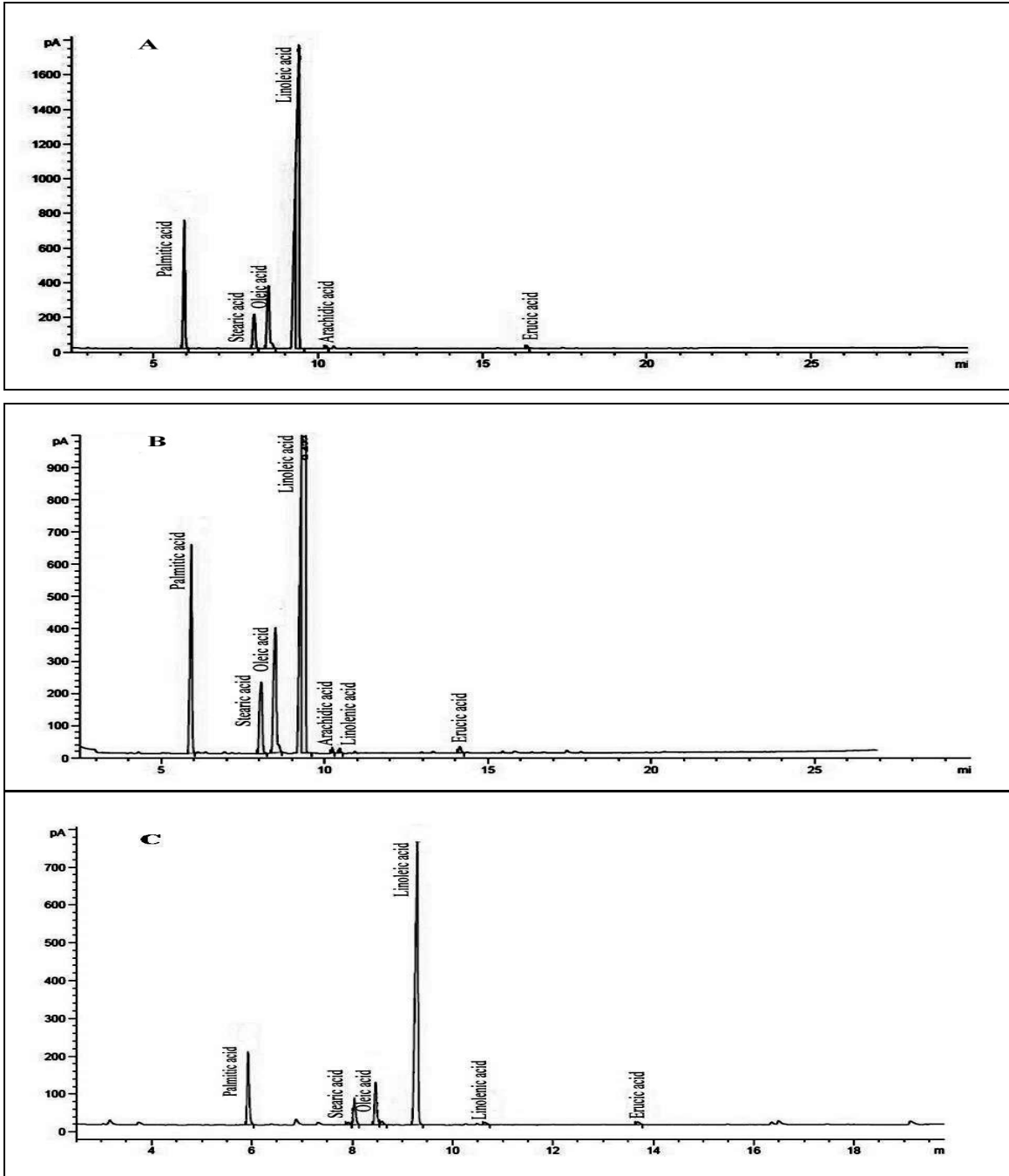


Fig. 2. GC Chromatogram for fatty acids profile of winter melon (*B. hispida*) seed oil; **A** (Cultivar 1), **B** (Cultivar 2), and **C** (Cultivar 3).

The concentration of major fatty acids as studied in the present work was noted to be almost comparable with other cucurbits seed oils. *Cucumeropsis manni* seed oil had a range of 15-24, 10-12.3, 9-18, and 42-61% of palmitic acid, stearic acid, oleic acid and linoleic acid, respectively (Badifu, 1991; Fokou *et al.*, 2009). *Cucurbita pepo* seed oil contained fatty acids compound within a range of 9.9-49.2% (palmitic acid), 4.87-11.2% (stearic acid), 17.0-47.0% (oleic acid), and 4.9-55.6% (linoleic acid) (Tsaknis *et al.*, 1997, El-Adawy & Taha, 2001, Nederal Nakic *et al.*, 2006; Nyam *et al.*, 2009). Palmitic acid (10.7-11.36%), stearic acid (7.04-9.00%), oleic acid (13.25-18.1%), and linoleic acid (59.6-68.3%) were detected as the most abundant fatty acids in *Citrullus lanatus* seed oils (El-Adawy & Taha, 2001, Milovanovic and Picuric-Jovanovic, 2005, Mariod *et al.*, 2009, Nyam *et al.*, 2009 and Baboli & Safe Kordi, 2010).

Phytosterols composition: The data for the qualitative and quantitative analysis of phytosterols analyzed in winter melon seed oils is shown in Table 5. The seed oils from the three tested cultivars of winter melon mainly consisted of β -sitosterol (54.62–60.50%), campesterol (15.10–18.50%), stigmasterol (11.00–14.30% and Δ^5 -avenasterol (6.40–8.14 %). A considerable amount of 28-isoavenasterol, clerosterol and 24-Methylenecholesterol with contribution of 0.95 to 1.20, 0.85 to 1.70 and 0.65 to 1.30% was also detected. A small level < 1.0% of stigmastanol, Δ^7 -avenasterol, and Δ^7 -campestanol was also present. The concentration of the main phytosterols components in the present analysis of winter melon seed oils was in agreement to those of cucurbit seed oils (Nyam *et al.*, 2009) and other common vegetable oils (Rossell, 1991). Differences in the composition of oils phytosterols in relation to regional, cultivar and crop species variations are reported in the literature (Norman, 1979, Rossell, 1991).

Table 5. Phytosterol composition (%) of winter melon (*B.hispida*) fruit seed oils.

Phytosterol	Cultivar 1	Cultivar 2	Cultivar 3
Stigmasterol	11.00 ± 0.50	12.92 ± 0.60	14.30 ± 0.55
24-Methylenecholesterol	1.30 ± 0.10	0.65 ± 0.10	1.00 ± 0.05
Clerosterol	1.05 ± 0.15	0.85 ± 0.10	1.70 ± 0.20
Campesterol	16.20 ± 0.35	18.50 ± 0.82	15.10 ± 1.00
β -Sitosterol	60.00 ± 0.50	54.62 ± 0.78	57.00 ± 0.67
Δ^5 -Avenasterol	6.40 ± 0.25	8.14 ± 0.55	7.90 ± 0.65
Δ^7 -Avenasterol	0.90 ± 0.15	0.88 ± 0.15	0.50 ± 0.10
Δ^7 -Campestanol	0.50 ± 0.08	ND	0.30 ± 0.10
28-Isoavenasterol	1.20 ± 0.20	0.95 ± 0.07	1.00 ± 0.20
Stigmastanol	0.43 ± 0.15	0.65 ± 0.10	0.81 ± 0.15

Means with different letters within the same row denote significant differences among cultivars ($p < 0.05$); Cultivar 1, round fruit; Cultivar 2; oval fruit; Cultivar 3, round (hybrid) fruit; ND; not detected

Conclusion

The proximate analysis of seeds and physicochemical attributes of the extracted seed oils were appraised for the three cultivars of winter melon (*B.hispida*) grown in Malaysia. The seeds from the tested cultivars were explored as a good source of oil, protein and fiber and thus could be consumed for dietary purposes. The extracted seed oils revealed the presence of high amount of linoleic acid (63.10-70.64%), placing these oils in the category of high-linoleic vegetable oils. A reasonable amount of valuable tocopherols and phytosterols with potential health benefits were also established. Besides, the examined seed oils also offered an acceptable level for other quality-oriented physicochemical characteristics. Presence of high amount of polyunsaturated fatty acid (linoleic acid), along with considerable amounts of tocopherol and phytosterol like beneficial components revealed the suitability and potential of these oils for edible and other functional food applications. Further investigation on the detailed antioxidant attributes and individual phenolics composition of the seed oils from Malaysian winter melon fruit is strongly recommended.

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