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Phytochemicals, nutritionals and antioxidant properties of miracle fruit *Synsepalum dulcificum*

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ABSTRACT

Synsepalum dulcificum, also called the miracle fruit, which has the sweet-inducing activity can be used as additives in food, medicine and cosmetic industries. Some selected chemical properties of miracle fruit including percentage by weight, total anthocyanin, phenolic and antioxidant content of different parts of miracle fruit as well as physicochemical analysis of seed oil, nutritional elements of fruit juice were determined in this study. The results showed that miracle fruit contains a large amount of vitamin C (40.1 mg/100 g fresh fruit weight (FW)), phenolic content (625.57 mg GAE/100 g FW), high antioxidant capacity (457.3 μmol Trolox/100 g FW) and low total sugar content (5.6 g/100 g FW), suggesting that the fruit is healthy for human consumption. According to its fatty acid composition and Triacylglycerol (TAG) profile, miracle fruit seed oil is rich in oleic and palmitic acid.

1. Introduction

The miracle fruit, *Synsepalum dulcificum*, is an evergreen shrub belongs to the *Sapotaceae* family, *Synsepalum* genus. Miracle fruit is described as a berry that can change sour to sweet taste. The pulp of miracle fruit contains miraculin, a glycoprotein, which can cause sour food to taste sweet while it is tasteless (Wong and Kern, 2011). The sweet inducing activity of miracle fruit could be exploited for use in food, medicine and cosmetic industries as sweeteners or additives (Wilken and Satiroff, 2012; Wong and Kern, 2011).

The miracle fruit plant is a shrub that grows up to 6.1 m high in its native habitat, but does not usually grow higher than 3 m in cultivation. The plant leaves are 5–10 cm long, 2–3.7 cm wide and glabrous below while the flowers are brown in colour. The ripened miracle fruits are red in color with about 2 cm long and they are clustered at the ends of the branches. Each fruit contains a thin layer of edible pulp surrounding an elongate-ovoid shape seed (Chen et al., 2012). The plant can be harvested twice a year, so the yield is stable. Nowadays, research on miracle fruit is focused on its miraculin content which has unique properties and characteristics. The pulp of miracle fruit, the only part of the fruit that contains miraculin, takes only 4.44% weight of the fresh fruit (Inglett and Chen, 2011). The components of other parts of the fruit are rarely investigated and the information related to these is not available in the literature.

Traditionally, deep colored fruits, vegetables or foods are recognized as more healthy to human body. The pigment components of fruits, famous for anthocyanin, may promote human health or lower the risk for disease (Lin and Tang, 2007). Moreover, phenolic compounds, containing anthocyanin, which are presence in fruits, vegetables, leaves, nuts, seeds, flowers, and barks, are an integral part of the human diet and are also taken intentionally as medicinal preparations (Sellappan et al., 2002). Furthermore, antioxidant which normally related to phenolic compounds, is considered to have the effect to improve the quality and nutritional value of food. At the same time, the search for antioxidants from natural sources has attracted increasing attention due to the widespread agreement of the potential health risks and toxicity of synthetic antioxidants such as BHA and BHT (Dudonne et al., 2009). The importance of antioxidant constituents of plant materials in the maintenance of health and protection from coronary heart disease

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and cancer is also raising interest among scientists, food manufacturers, and consumers as the trend of the future is moving toward the development of functional food with specific health effects (Kähkönen et al., 1999).

Many kinds of fruit seed belong to *Sapotaceae* family such as star apple, mamey sapote have been studied. These large grain seeds have oils that exhibit a significantly high content of unsaturated or essential fatty acids, and show great potential for industrial usage. Unfortunately, there is limited understanding of miracle fruit seed and its oil (Ariffin et al., 2009). Attempt to separate the lipids from miracle fruit using thin-layer chromatography have been reported (Guney and Nawar, 1977) and no other information related to this could be found in the literature. It is important to characterize miracle fruit due to its health potential. In this study, some physicochemical properties of miracle fruit including percentage weight and nutritional elements (ash, crude protein, fat, total dietary fiber, carbohydrate contents and energy value) were determined. The total anthocyanin, phenolic, and antioxidant content of different parts of miracle fruit were also determined. For the sake of its potential usage, the melting and crystallizing behavior, fatty acid composition and triacylglycerol composition of miracle fruit seed oil were also analyzed. All these work were done to add to the limited amount of information available on miracle fruit.

2. Materials and methods

2.1. Miracle fruit

Fresh ripened miracle fruits were obtained from a local farm (Nilai Nursery, Nilai, Malaysia) as shown in Fig. 1. The skin and seed were separated from the pulp of the miracle fruits using a knife, and all of the skin, pulp and seed were freeze dried and ground into fine powder. The powder was kept at −30 °C prior to extraction.

2.2. Physicochemical analysis

Dry matter, crude protein, ash, total sugar and total dietary fiber were determined according to the procedures outlined by Horwitz (2000). Determination of carbohydrate followed the method described by Tee et al. (1997). The fat was determined based on FAO Food and Nutrition paper reported by Witty (1998). Weight percentages of different parts of miracle fruit were also determined according to the method of Inglett and Chen (2011).

Vitamins A and C were determined using high performance liquid chromatography (HPLC) (Aglient 1200, Waters, Milford, Massachusetts) with a DAD detector (UV). Vitamin A was determined using a C18 column (Phenomenex KINETEX, 2.6 μm, 4.6 mm × 150 mm) with beta carotene as a standard (Sigma). A mixture of acetonitrile: methanol: ethyl acetate (88:10:2) was used as a mobile phase with a flow rate of 1 mL/min. On the other hand, vitamin C was determined using a C18 column (Zorbax ODS, 5 μm, 4.6 mm × 150 mm) with ascorbic acid as a standard (Sigma). The mobile phase was a mixture of acetonitrile: ammonium acetate: water (25:25:50) with a flow rate of 1 mL/min. For both vitamins, the detector was set at 30 °C.

2.3. Determination of anthocyanin, total phenolic, and antioxidant contents

2.3.1. Sample preparation

The extraction method followed the one of Velioglu with minor modification (Velioglu et al., 1998). Briefly, 4 g ground samples were extracted with 100 mL of 80% aqueous methanol at room temperature using an orbital shaker for 120 min. The mixture was subsequently filtered using Whatman filter paper (No.5), and the filtrate was centrifuged at 6000 g for 10 min. The solvent supernatant was then transferred to tubes. All extracts were stored at −30 °C prior to use in analyses.

2.3.2. Determination of total antioxidant

The total antioxidant in the extracts of skin, pulp and seed was determined according to the DPPH radical scavenging activity test as described by Şensoy et al. (2006) where the absorbance was measured at 515 nm. The calibration curve was determined using trolox (6-hydroxy-2,5,7,8-tetramethyl-chroman-2-carboxylic acid), as a standard with a range of concentration from 0.01 mM to 1.25 mM. The results were presented using trolox equivalents as mmol/L. The results were then converted to μM trolox/100 g of fresh fruit weight and μM trolox/g of dry fruit weight.

2.3.3. Determination of phenolic content

The phenolic content in the extracts of skin, pulp and seed were determined following the method as described by Zhou and Yu (2006). The reaction mixture contained 50 μL of extracts, 250 μL of the Folin-Ciocalteu reagent (Sigma, America). The absorbance was measured at 765 nm. Gallic acid was used as standard and the results were presented as mg of gallic acid equivalents per 100 g fresh fruit weight (GAE/100 g fresh fruit weight (FW)).

2.3.4. Determination of total anthocyanin content

The total anthocyanin content was determined by the pH-differential method as described by Giusti and Wrolstad (2001). Anthocyanin pigments transform reversible structure with a change in pH proven by strikingly different absorbance spectra. The colored oxonium form predominates at pH 1.0 and the colorless hemiketal form dominates at pH 4.5. The pH-differential method is based on this reaction, and permits accurate and rapid measurement of the total anthocyanins.

Briefly, 1 mL of sample was transferred into 10 mL volumetric flask for the preparation of sample with two dilutions. The volume of the first one was adjusted with potassium chloride buffer pH 1.0 while the other one the volume was adjusted with sodium acetate buffer (pH 4.5). The dilutions were equilibrated for 15 min and the absorbance of each sample dilution was measured at 510 and 700 nm, against a blank cell filled with distilled water. The absorbance of the diluted sample (A) was calculated according to Eq. (1):

$$A = (A_{510nm} - A_{700nm})_{pH1.0} - (A_{510nm} - A_{700nm})_{pH4.5} \quad (1)$$

The anthocyanin pigment concentration in the original sample was calculated using Eq. (2):

$$\text{Concentration of anthocyanin}\left(\frac{\text{mg}}{\text{L}}\right) = (A \times MW \times DF \times 1000)/(\varepsilon \times 1) \quad (2)$$

where MW is the molecular weight, DF is the dilution factor, and ε is the molar absorptivity. The pigment content was calculated as cyanidin-3-glucoside, where the values of MW and ε used in the calculation was 449.2 and 26,900, respectively. The value was then converted to mg of total anthocyanin/100 g fresh fruit weight according to Eq. (3).

$$\text{Total anthocyanin/100g fresh fruit} = \frac{\text{concentration of anthocyanin}\left(mg/mL\right) \times \text{volume of solution}\,(mL)}{\text{concentration of skin in solution}\left(g/mL\right) \times \text{volume of solution}\,(mL) \times 1000} \quad (3)$$

2.4. Characterization of miracle fruit seed oil

The miracle fruit seed oil (MFSO) was characterized by Triacylglycerol (TAG) profile using high performance liquid

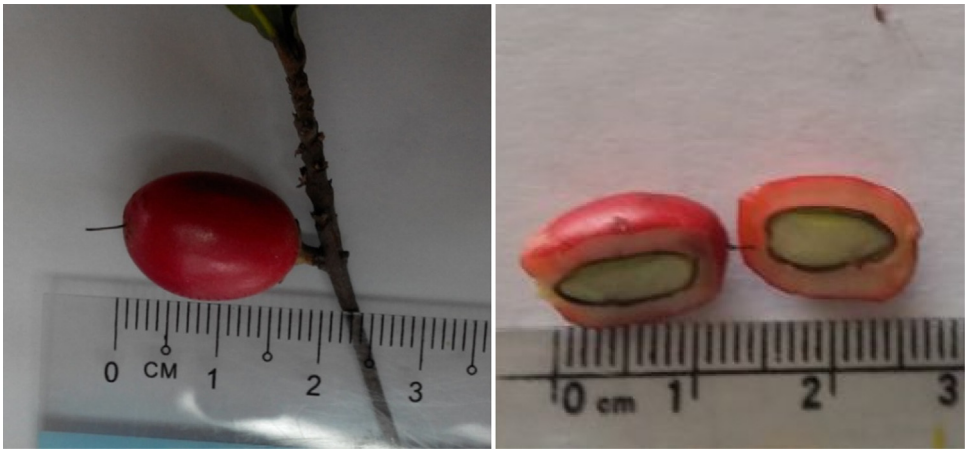

Fig. 1. Miracle fruit, its skin, pulp and seed.

chromatography (HPLC), fatty acid composition using gas chromatography (GC) and thermal behavior with differential scanning calorimetry.

2.4.1. Seed oil extraction

After the removal of the seed coating, the oil sample was extracted from the crushed seed kernels by Soxhlet extractor with petroleum ether (Sigma-Aldrich, America), following the method described by Abu-Arabi et al. (2000) with some modifications. Leaching was carried out at the boiling point of selected solvent for 8 h. The extracted phase (oil and solvent) was then distilled using a rotary evaporator with a vacuum pump to ensure complete removal of the solvent. The oil sample was stored at −20 °C prior to use in the analysis.

2.4.2. GC analysis of seed oil of miracle fruit

The MFSO were analysed as fatty acid methyl ester (FAMEs), prepared using the method as described by O'fallon et al. (2007) with some modifications. After the esterification of sample, hexane was added and vortexed for 5 min. The hexane layer containing FAME was placed in chromatography vial to be analyzed using gas chromatography or kept at −20 °C for further analysis.

FAME was analyzed with Agilent 7890A (Santa Clara California, USA), GC equipped with Flame Ionization Detector (FID). Separation was carried out using capillary column BPX70 70% Cynopropyl Polysilphenylene-siloxane (SGE Analytical Science, Ridgewood Victoria, Australia), 30 m in length, with internal diameter of 0.32 μm and nitrogen as carrier gas with a flow rate fixed at 5.7 mL/min. The injector and detector temperature were set at 250 and 280 °C, respectively. The split ratio used was 15:1. Oven temperature was programmed as follows: holding at 1 °C for 4.6 min, 100–170 °C at a rate of 10 °C/min, 170–230 °C at a rate of 1.5 °C/min and hold at 230 °C for 7 min, 230–250 °C at a rate of 30 °C/min and hold at 250 °C for 1 min.

2.4.3. Determination of thermal behaviour of seed oil of miracle fruit

The crystallization and melting thermograms of the MFSO was determined using Perkin Elmer DSC 8000 (Waltham Massachusetts, USA), according to the method of Lee et al. (2013b). Firstly, the sample was heated to 80 °C and held for 10 min to destroy any crystal memory contained in the sample. Thereafter, it was cooled to −70 °C at a rate of 5 °C/min and held at −70 °C for 10 min to obtain the crystallization thermogram. The sample was then heated from −70 °C to 80 °C at a rate of 5 min/min and held at 80 °C for 10 min. An effort was made to ensure that all samples were of the same weight but not identical. All determinations were carried out with duplicate analysis.

2.4.4. Acylglycerol composition

Reacted samples were analysed for its acylglycerol composition using Alliance e2695 Separation Modules High performance liquid chromatography (HPLC) (Waters, USA) coupled with evaporative light scattering (ELS) 2424 detector (Waters, USA). Sample was prepared by adding 5% w/v oil sample in acetone and 10 μL of sample was injected into Purospher®Star RP-18e column (5 μm, 250 mm × 4 mm, Merck, Germany). Mobile phase set gradient of acetone (A) and acetonitrile (B) with a flow rate of 1 mL/min and programmed as follow; 0 min: 90% B, 8 min: 85% B, 40 min: 10% B, 50 min: 90% B, 52 min: 90% B. The drift tube temperature, nebulizer power and gas pressure of ELS detector were set at 45 °C, 60% (heating) and 35 psi, respectively.

2.4.5. Statistical analysis

All experiments and analyses were replicated three times for each sample. The results were presented as mean ± standard deviation (SD). The differences of the physicochemical properties of fruits harvested from several plants at different times were statistically analyzed using one-way ANOVA in SPSS 17.0. The significance of differences between the means was determined using the Tukey's test ($p < 0.05$). In most cases, the mean values for the different fruits harvested from different plants at different times were not significantly different (data not shown).

3. Results and discussion

3.1. Proximate chemical analysis

The weight percentage of pulp, seeds and skin in the miracle fruit are summarized in Table 1. The seeds occupied 64.11% of lyophilized fruit, while skin and pulp take 15.47% and 20.42%, respectively. The data of dry basis were comparable to those reported by Inglett and Chen (2011). However, the water content in the fresh fruit (65.33%) obtained in this study was lower than that reported by the same researchers (75.22%). The edible part of miracle fruit is low because of high proportion of seed.

The proximate chemical composition of miracle fruit fleshes are shown in Table 1. The total sugar, total dietary fiber, ash, carbohydrate, vitamin A and vitamin C were found to be 5.6 g/100 g FW, 12.5 g/100 g FW, 1.0 g/100 g FW, 22.5 g/100 g FW, 37.3 μg/100 g FW, and 40.1 mg/100 g FW, respectively. It is interesting to note that the fleshes of miraculous fruit did not contain any fat. It is well

Table 1
The percentage of water, pulp, seeds and skin contributed to fresh weight and freeze-dried weight of miracle fruit and proximate chemical composition of miracle fruit fleshes.

	Weight% (wet basis)	Weight% (dry basis)
Seeds	22.22 ± 0.68	64.11 ± 1.05
Skin	5.36 ± 0.42	15.47 ± 0.59
Pulp	7.08 ± 0.30	20.42 ± 1.52
Water	65.33 ± 0.75	–
Total	100 ± 2.15	100 ± 3.16

Miracle fruit fleshes	
Fat (g/100 g fresh fruit weight).	0 ± 0.00
.Total sugar (g/100 g fresh fruit weight)	5.6 ± 0.09
.Total dietary fibre (g/100 g fresh fruit weight)	12.5 ± 1.32
.Ash (g/100 g fresh fruit weight)	1 ± 0.06
.Carbohydrate (g/100 g fresh fruit weight)	22.5 ± 1.64
.Vitamin A (μg/100 g fresh fruit weight)	37.3 ± 0.32
.Vitamin C (g/100 g fresh fruit weight)	40.1 ± 0.86

Data are presented as mean values; ±standard deviation (SD) of triplicate values.

known that fruits are the main sources of vitamin C. The vitamin C content in star apple, the other *Sapotaceae* family fruit, was only 0.16 mg/g (Kubola et al., 2011). The vitamin C content in miracle fruit is close to that in *Citrus* fruits (varies from 32.8 mg/100 g FW to 42.6 mg/100 g FW) (Isabelle et al., 2010), and kiwi fruit (55 mg/100 g FW) (Nishiyama et al., 2004), which are commonly thought to be the excellent sources of vitamin. Moreover, as the potential functional food in diet for the patients of diabetes and obesity, total sugar content in miracle fruit is low, which is lower than berries (Eyduran, 2006). Total sugar content of miracle fruit is similar to raspberry (5.58 g/100 g FW), which is considered as fruit with low-sugar content.

3.2. Determination of antioxidant phenolic, and anthocyanin content

Table 2 demonstrates the total content of antioxidant, phenolic, and anthocyanin in different parts of miracle fruit. Phenolic content (73.79 mg GAE/g) was found in the skin, which was significantly larger than that in the pulp (32.46 mg GAE/g) and seeds (13.52 mg GAE/g). The study confirmed that phenolic substances are concentrated in skin. Because of the different weight ratios, skin contributed 395.77 mg GAE/100 g FW, following by the seeds and pulp, contributed 300.51 mg GAE/100 g FW and 229.8 mg GAE/100 g FW, respectively.

Phenolic content in flesh, the total content in pulp and skin, was 625.57 mg GAE/100 g FW. This value was almost double than that reported by Inglett and Chen (2011) (335.85 mg GAE/100 g FW). The differences in phenolic content reported by different researchers may be due to the variety of fruit itself, cultivation climate, ripening stage, harvesting time and the loss in extraction procedure. In comparison to berries, which usually considered as fruit rich in phenolic content, such as blackberry (435 mg GAE/100 g FW), blueberry (348 mg GAE/100 g FW) or strawberry (294 mg GAE/100 g FW) (Heinonen et al., 1998), total phenolic content in the flesh of miracle fruit is much higher. The phenolic content of miraculin was comparable with longan (633.9 mg GAE/100 g FW) and kiwifruit (685.9 mg GAE/100 g FW), but higher than the most common fruits studied by Isabelle et al. (2010). Considering the edible part in miracle fruit is quite little, the phenolic content in flesh is very high. Phenolic compounds have been shown to inhibit *in vitro* oxidation of human low-density lipoprotein (LDL), suggesting that miracle fruit could be good for health (Heinonen et al., 1998).

Antioxidant content of miracle fruit is summarized in Table 2, presented as μmol Trolox/g dry fuit weight and μmol Trolox/100 g FW, respectively. Antioxidant in the skin was 50.27 μmol Trolox/g dry fuit weight, which was approximately two times of that in the pulp and four times in the seeds. However, due to the weight ratio, the seeds take the most antioxidant content (327.85 μmol Trolox/100 g FW), antioxidant content in the skin and pulp was 269.62 μmol Trolox/100 g FW and 187.68 μmol Trolox/100 g FW. Thus, the seed may have the potential to be the good source of antioxidant. With the edible part, antioxidant content in the skin and pulp was calculated as 457.3 μmol Trolox/100 g FW. Referring to the fruits studied by Vasco et al. (2008), antioxidant capacity of miracle fruit is higher than tomato (80 μmol Trolox/100 g FW), sweet pepino (30 μmol Trolox/100 g FW), mango (310 μmol Trolox/100 g FW), passion fruit (50 μmol Trolox/100 g FW) and some other fruits. High antioxidant capacity of miracle fruit indicates that it can be used as supplements to improve human health. It is well known that dietary antioxidants can stimulate cellular defenses and help to prevent cellular components against oxidative damage (Dudonne et al., 2009).

The total anthocyanin content of miracle fruit is shown in Table 2 (11.04 mg/100 g FW), which may be possibly responsible for the red color of the fruit. The anthocyanin content was similar to the content reported by Buckmire and Francis (1976) (14.3 mg/100 g FW). This researcher also claimed that the major composition of anthocyanin in miracle fruit was cyanidin-3-monogalactoside, which contributed 70.7% of the pigment. Anthocyanin was easy to decompose during the extraction process. The variation in anthocyanin content may be due to the loss of anthocyanin during the extraction. However, anthocyanin content in miracle fruit is relatively low as compared to many other common fruits, such as blackberry (1/15), kiwifruit (1/10) and strawberry (1/3) (Moyer et al., 2002).

It is notable that the antioxidant capacity evaluated in this study was apparently higher than that reported by Inglett and Chen (2011), which claimed the antioxidant capacity (196.06 μmol Trolox/100 g) in the flesh of miracle fruit using the same determination method. Thus, antioxidant capacity may significantly affected by the fruit itself. Moreover, evaluation of the total antioxidant capacity of fruits, vegetables, and other plant products cannot be performed accurately by any single method due to the complex nature of phytochemicals and different detection methods may deliver variation in results (Dudonne et al., 2009). High vitamin C and phenolic content may together contribute to high performance of antioxidant capacity of miracle fruit. The data presented in this study are in agreement with some other reports in the literature (Duhita et al., 2009; Lee et al., 2003). However, the relatively low concentration of anthocyanin content indicates that the antioxidant content in miracle fruit is mainly associated with its phenolic content.

3.3. Proximate analysis of seed oil of miracle fruit

The weight of seed oil from miracle fruit was 6% of the total weight of the freeze-dried seeds. It was light green in color and viscous at room temperature. It also becomes solid when kept at temperature below 4 °C. According to Guney and Nawar (1977), lipids took 10.15% of the dry weight of miracle fruit seeds.

3.3.1. Melting and crystallizing behavior

Melting and crystallizing, are two physical events, normally used to characterize thermal behavior of oil samples which can be determined using DSC. The process of melting and crystallization require intake and release of thermal enthalpy. The heating and cooling profiles for the seed oil extracted from the miracle fruit are presented in Fig. 2. In the melting thermograms, 4 endothermic peaks were observed at −0.1 °C, 9.30 °C, 17.22 °C and 27.35 °C. In the cooling thermograms, 3 peaks were observed at −0.78 °C, 9.63 °C and 23.8 °C.

Table 2
Total anthocyanin, phenolic, flavonoid and antioxidant content in different parts of miracle fruit.

Component	Seeds	Pulp	Skin	Total
Anthocyanin (mg/100 g fresh fruit weight)	–	–	11.04 ± 0.1	11.04 ± 0.1
Phenolic (mg/100 g fresh fruit weight)	300.51 ± 11.78	229.80 ± 5.45	395.77 ± 4.40	926.08 ± 21.63
Antioxidant (μmol Trolox/100 g fresh fruit weight)	327.85 ± 24.89	187.68 ± 21.59	269.62 ± 17.00	785.15 ± 63.68
Phenolic (mg/g)	13.52 ±	32.46 ± 0.77	73.79 ± 0.82	
Antioxidant (μmol Trolox/g dry fruit weight)	14.75 ± 1.12	26.51 ± 3.05	50.27 ± 3.17	

Data are presented as mean values; ±standard deviation (SD) of triplicate values.

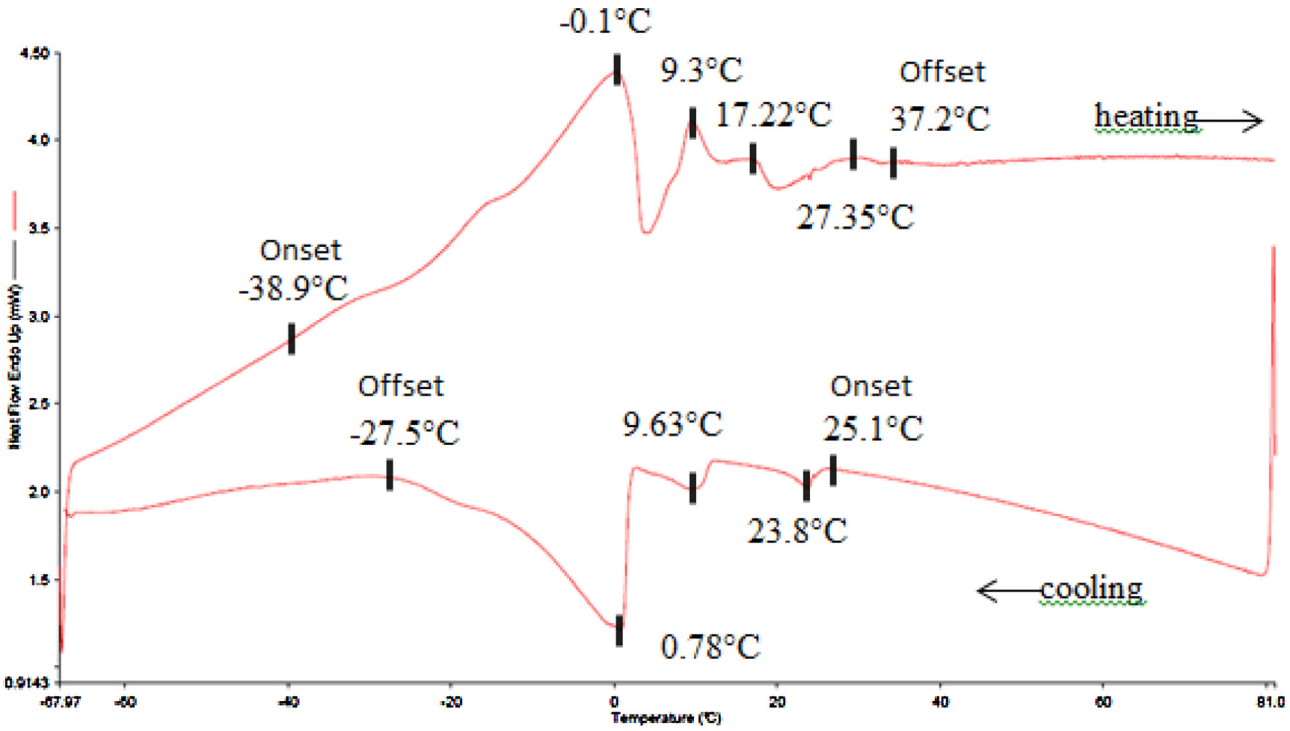

Fig. 2. Differential scanning calorimetry (DSC) profile of miracle fruit seed oil.

3.3.2. Fatty acid composition

From the FA chromatogram, 10 different fatty acids (FA) were identified in miracle fruit seed oil (MFSO) and the results are summarized in Table 3. They could be divided into four categories: saturated; monounsaturated; polyunsaturated of the n-6 family and polyunsaturated of the n-3 family (Parker et al., 2003). The total saturated fatty acids were 41.4% in MFSO while the major components in that group were palmitic acid (33.4%) and stearic acid (7.0%). The total unsaturated fatty acids were 52.7% of which, the monounsaturated and polyunsaturated fatty acid accounted for 37.4% and 15.3%, respectively. The major unsaturated fatty acids were oleic acid (37.2%), linoleic acid (14.2%) and linolenic acid (1.1%). Small amount of lauric acid (0.6%), arachidic acid (0.2%) were found in the oil. Thus, MFSO could be considered as oleic-palmitic acid rich oil. All the identified fatty acids accounted for 94.1% of total content in the sample. High levels of fatty acid was also reported in raspberry seed oil (93.8%) (Oomah et al., 2000) and (95.7%–95.9%) (Winton and Winton, 1939). In comparison to other plant oils, among of them, the major fatty acid contents of MFSO was similar to palm oil, which had oleic acid: linoleic acid: palmitic acid at a percentage ratio of 39.2: 10.1: 44 (Table 4). In addition, MFSO contains higher proportion of palmitic acid, while in red raspberry, marionberry, boysenberry and blueberry, the palmitic acid content was only 1.3%, 3.3%, 4.2% and 5.7%, respectively (Parry et al., 2005). In contrast, linoleic acid and linolenic acid were the major seed oil FA from these fruits, where the content was 85.4%, 78.6%, 73.3% and 68.6%, respectively. In the seed oil of the above mentioned berries, more than 90% of the fatty acids were in the unsaturated form while in MFSO, the amount was only half.

Miracle fruit seed oil (MFSO) may have the same potential application as palm oil producing edible oil due to its similar fatty acid composition. Palmitic acid, stearic acid, oleic acid and linoleic acid made up 91.8% in MFSO. Oleic acid is the most common monounsaturated acid and also the most common acid produced in nature. Stearic acid was reported to be as effective as oleic acid in the role of lowering the plasma cholesterol level (Gunstone et al., 2012). Linoleic acid could produce a mixture of conjugated linoleic acid isomers, which are generally found in trans (t)/cis (c), c/t and t/t forms, containing the 9c11t and 10t12c under controlled conditions. These isomers have potential uses in modifying body composition and as anticancer agents (Gunstone et al., 2012). Uptake of polyunsaturated fatty acid and monounsaturated fatty acid increases blood pressure, while the ingestion of saturated fats causes the opposite effect (Mutanen et al., 1992). The lauric acid content is only 0.6% in MFSO, which is known to raise plasma lipid saturated fatty acid levels (Edem, 2002).

3.3.3. Triacylglycerol (TAG) composition analysis

HPLC chromatogram shows the triacylglycerol composition in the seed oil (Table 4 and Fig. 3). The MFSO contained 77% triacylglycerol. The results indicated that the most prominent monounsaturated TAG was palmitic acid: oleic acid: palmitic acid

Table 3
Fatty acid composition of miracle fruit seed oil and other plant oils.

Fatty acid	MFSO	Palm oil (Edem, 2002)	Palm kernel oil (Edem, 2002)	Jatropha curcas seed oil (Akbar et al., 2009)
Caproic 6:0	–	–	0.2	–
Caprylic 8:0	–	–	3.3	–
Capric 10:0	–	–	3.5	–
Lauric 12:0	0.6	0.2	47.8	–
Myristic 14:0	0.1	1.1	16.3	0.1
Palmitic 16:0	33.4	44.0	8.5	14.2
Palmitoliec 16:1	0.2	–	–	0.7
Margaric 17:0	0.1	–	–	0.1
Stearic 18:0	7.0	4.5	2.4	7.0
Oleic 18:1	37.2	39.2	15.4	44.7
Linoleic 18:2	14.2	10.1	2.4	32.8
Linolenic 18:3	1.1	0.4	–	0.2
Arachidic 20:0	0.2	0.1	0.1	0.2
Saturates	41.4	49.9	82.1	21.6
Monounsaturated	37.4	39.2	15.4	45.4
Polyunsaturated	15.3	10.5	2.4	33.0

Fatty acid	Coconut oil (Edem, 2002)	Soybean oil (Edem, 2002)	Sunflower oil (Edem, 2002)	Groundnut oil (Edem, 2002)	Cotton seed oil (Edem, 2002)	Corn oil (Edem, 2002)
Caproic 6:0	0.5	–	–	–	–	–
Caprylic 8:0	8.0	–	–	–	–	–
Capric 10:0	6.4	–	–	–	–	–
Lauric 12:0	48.5	–	–	–	Trace	–
Myristic 14:0	17.6	0.1	–	0.1	0.8	–
Palmitic 16:0	8.4	11.0	–	11.0	23.7	6.5
Palmitoliec 16:1	–	–	–	–	–	–
Margaric 17:0	–	–	–	–	–	–
Stearic 18:0	2.5	4.0	4.5	3.1	2.6	2.2
Oleic 18:1	6.5	23.4	21.1	48.5	18.4	27.5
Linoleic 18:2	1.5	53.2	66.2	31.4	53.0	57.0
Linolenic 18:3	–	7.8	–	–	0.1	0.9
Arachidic 20:0	–	–	0.3	1.5	0.3	0.1
Saturates	91.9	15.1	11.3	16.3	27.4	14.5
Monounsaturated	6.5	23.4	21.1	48.5	18.4	27.5
Polyunsaturated	1.5	61.0	66.2	31.4	53.1	57.9

Table 4
Triacylglycerol (TAG) profile of miracle fruit seed oil and some other plant oils.

TAG species	Miracle fruit seed oil (MFSO)	Palm oil (Lida et al., 2002)	Sunflower oil (Lida et al., 2002)	Soybean oil (Lida et al., 2002)	Sesame oil (Lee et al., 2013a)	Canola oil (Lida et al., 2002)
DAG	22.5	–	–	–	–	–
LLnL	–	–	–	4.0	–	–
LLL	–	–	27.2	25.4	9.6	1.2
OLnL	–	–	–	2.7	–	1.3
PLnL	–	–	–	0.8	–	–
OLL	0.5	0.4	29.5	26.5	29.4	4.8
OLnO	–	–	–	–	–	4.1
PLL	1.8	1.2	9.6	13.8	4.4	0.6
OLO	1.2	1.5	11.0	9.9	26.6	25.4
PLO	8.5	8.9	10.0	7.9	9.1	1.7
PLP	7.5	9.2	0.6	3.0	1.8	–
MPP	0.5	0.2	–	–	–	–
OOO	2.4	3.9	3.0	2.9	10.0	56.8
POO	19.0	23.3	3.5	1.3	4.2	2.7
POP	25.1	30.2	0.5	1.8	3.0	0.4
PPP	1.4	6.7	0.8	–	–	–
SOO	1.3	2.9	1.1	–	1.8	0.9
POS	6.6	6.7	0.4	–	–	–
PPS	0.8	1.1	0.4	–	–	–
PSS	0.5	–	2.4	–	–	–
Others	22.9	3.8	0.0	0.0	0.1	0.1

L, linoleic acid; Ln, linolenic acid; O, oleic acid; P, palmitic acid; M, myristic acid; S, stearic acid. Others include DAG and/or unidentified TAG. For MFSO, the DAG is 22.5%.

(POP) (25.1%), followed by palmitic acid: oleic acid: oleic acid (POO) (19.0%), palmitic acid: linoleic acid: palmitic acid (PLP) (7.5%), oleic acid: oleic acid: oleic acid (OOO) (2.4%), Polyunsaturated TAG that has been detected were palmitic acid: oleic acid: linoleic acid (POL) (8.5%), oleic acid: oleic acid: linoleic acid (OOL) (1.2%), palmitic acid: linoleic acid: linoleic acid (PLL) (1.8%), stearic acid: oleic acid: oleic acid (SOO) (1.3%), palmitic acid: oleic acid: stearic acid (POS) (6.6%). Saturated TAG found was palmitic acid: palmitic acid: palmitic acid (PPP), accounting for 1.4%. palmitic acid: palmitic acid: stearic acid (PPS), palmitic acid: stearic acid: stearic acid (PSS), oleic acid: linoleic acid: linoleic acid (OLL) and myristic acid: palmitic acid: palmitic acid (MPP) were also detected in micro

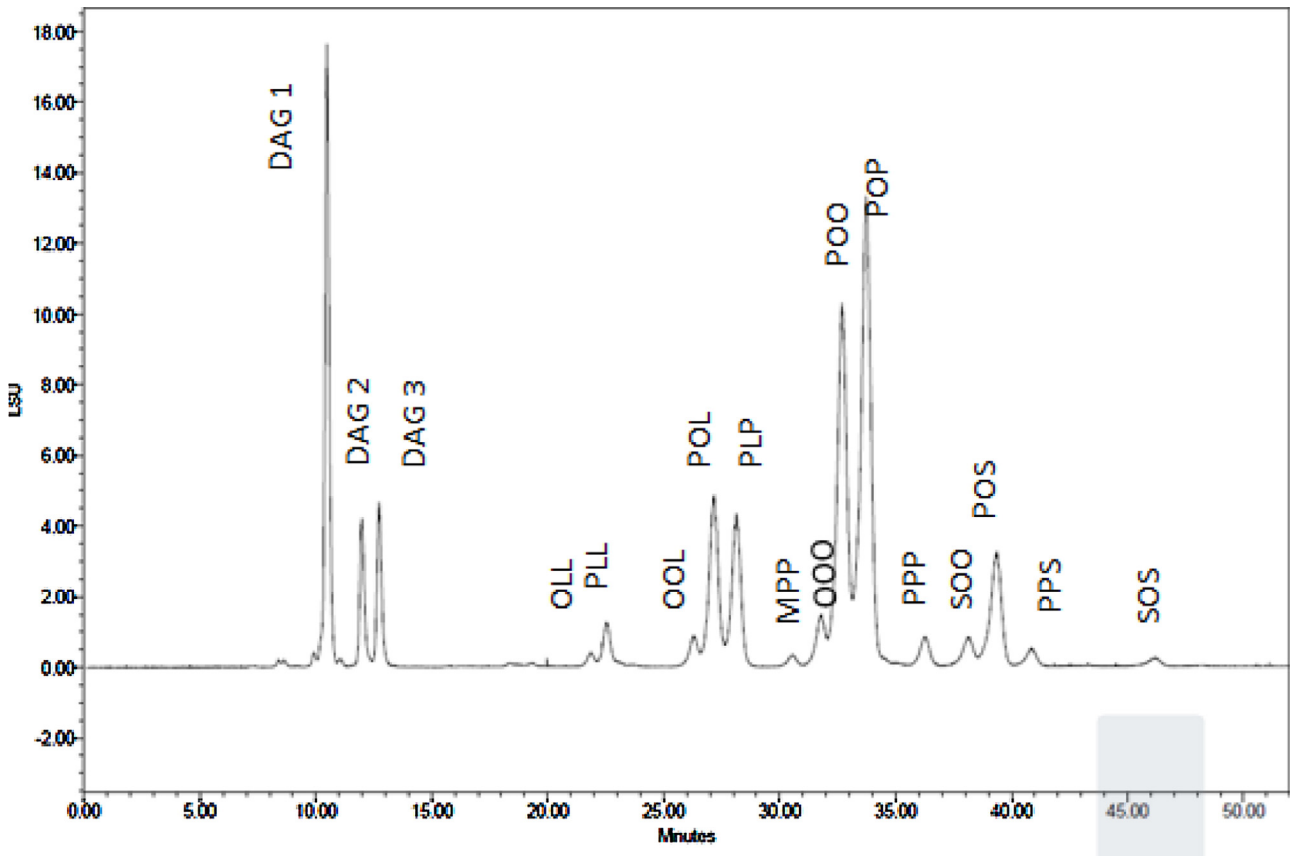

Fig. 3. Triacylglycerol (TAG) profile of miracle fruit seed oil (MFSO).

quantities (<1%). All the triacylglycerol species identified in MFSO were either monounsaturated or polyunsaturated forms with the total degree of unsaturation of 74.3%. What was notable, the graph also demonstrates there was a large amount of diacylglycerol (DAG) in MFSO, which was around 22.4%.

Comparison of TAG profile of MFSO and some other oils is shown in Table 4. The TAG profile of miracle fruit seed oil is extremely similar to the one of palm oil. Unlike sunflower oil, soybean oil and sesame oil, which were dominated by oleic acid: linoleic acid: oleic acid (OLO) and OLL in TAG profile, MFSO contained a lot of POO and POP. The data also suggests that MFSO has similar amounts of oleic acid and palmitic acid according to the peak area presented in the HPLC chromatography, similar to fatty acid composition analysis as described above.

4. Conclusion

For giving a fully understanding of potential utilization of miracle fruit, some physico-chemical properties of this fruit including percentage weight and nutritional elements (ash, crude protein, fat, total dietary fiber, carbohydrate and energy), total anthocyanin, phenolic, and antioxidant content of different parts of miracle fruit were demonstrated. The melting and crystallizing behavior, fatty acid composition and triaclglycerol composition of miracle fruit seed oil were also presented. Although the edible part of miracle fruit is not very much (12.44%), the flesh of miracle fruit contains a large amount of vitamin C (40.1 mg/100 g FW), phenolic content (625.57 mg GAE/100 mg FW) which gave high antioxidant capacity (457.3 μmol Trolox/100 g FW). The seed of miracle fruit is also a good source of antioxidant because of a large weight ratio. Moreover, the total sugar content in the flesh of miracle fruit is low (5.6 g/100 g FW), indicating that it may be healthy for human consumption, especially as sweeteners for the patients suffering from diabetes and obesity. The yield of oil from seeds of miracle fruit is 6%, the oil is green, viscous and fluid in room temperature. Fatty acid composition analysis found 10 fatty acids in the seed oil, the major components are oleic acid, palmitic acid and linoleic acid, as 37.2: 33.4: 14.2 (%). TAG profile of the seed oil shows the major components are POO (19%) and POP (25.1%), both fatty acid composition and TAG profile indicates miracle fruit seed oil is oleic-palmitic oil, highly similar to palm oil, suggesting that miracle fruit seed oil (MFSO) may have the same or analogous applications of palm oil.

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