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Fatty acid, triacylglycerol composition and antioxidant properties of date seed oil

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Abstract

Oil is one of the major components of date seed alongside dietary fibre, carbohydrate, protein, moisture and ash. Therefore, the present work focused on the extraction of oil from five varieties of date seed using Soxhlet extraction method and subsequently characterised their physicochemical and antioxidant properties accordingly. Oil extracted from the seeds ranged between 8 to 9.8%, whereas the iodine values were between 48.7 to 55.5 g I2/100g. Furthermore, oleic and lauric acids were revealed as the main fatty acids present in the date seed oil, with LaOO (La: lauric acid; O: oleic acid) as the main triacylglycerol. The total phenolic content in the oil ranged from 7.96 to 17.72 mg GAE/g oil, while the antioxidant activity, expressed as EC_{50}, ranged from 5.17 to 17.18 mg/mL. Additionally, the highest reducing activity was observed at 4mg/mL. Hence, oil characteristics are dependent on the type of date, thus indicating that different potential applications may be suggested.

Introduction

Date (*Phoenix dactylifera* L.) is an important fruit in some countries as a source of nutrition and economic growth (Al-Qarawi *et al.*, 2003; Awad, 2007; Briones *et al.*, 2011). About 11 to 18% of its weight is derived from the seed alone (Besbes *et al.*, 2004a; Nehdi *et al.*, 2010; Amira *et al.*, 2011). To date, there are more than 60 varieties of date fruit reported around the world (Bendiab *et al.*, 1998; Al-Shahib and Marshall, 2003a; Al-Shahib and Marshall, 2003b; Al-Farsi *et al.*, 2007; Habib and Ibrahim, 2009).

The Food and Agriculture Organization of the United Nations (FAO, 2017) reported that in 2014, the total world production of date fruits exceeded 7.5 million tons, thus rendering approximately more than 1 million tons of date seeds produced in that particular year alone. Based on information available from the literature, date seeds have been traditionally used as either animal feed or ground and roasted for it to serve as a caffeine-free coffee substitute (Rahman *et al.*, 2007). Otherwise, they are generally classified as waste by the date fruit industry, and thrown away.

Previous studies (Al-Shahib and Marshall, 2003a; Al-Shahib and Marshall, 2003b; Besbes *et al.*, 2004a; Habib and Ibrahim, 2009; Nehdi *et al.*, 2010; Al Juhaimi *et al.*, 2012) found that oil is present in date seeds in a moderate amount, with different varieties of date seed displaying varying amount and

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characteristics of such oil. Furthermore, the presence of antioxidants in date seed oil also elevated its beneficial characteristics. Antioxidant is known as a protective element against several diseases like coronary heart disease and cancer, which are related to oxidation in the human body. Oxidation of free radicals results in damages of bio-molecules like DNA, proteins, and lipids, which will then result in the development of several diseases (Chung *et al.*, 1998; Cadenas and Davies, 2000). Such process can be prevented by the presence of antioxidant, as it will inactivate and detoxify the free radicals (Tepe *et al.*, 2005). Many studies have proven that plants are a good source of natural antioxidants, whereby such compounds may be directly isolated from the plant parts (e.g. leaf) or by extracting from the plant oil, as most antioxidants are oil-soluble. However, different types of plants typically present varying compositions. Thus, the present work was aimed to determine the total oil content in five varieties of date seed, as well as analysing the physicochemical and antioxidant properties of the respective oils.

Materials and methods

Sample preparation

Five varieties of date (Mabroum, Mariami, Rotab, Safawi, Sufri) were obtained from a local importing company in Selangor, Malaysia. The Mariami and Rotab varieties originated from Iran, while Mabroum, Safawi and Sufri are typically sourced from Saudi Arabia. The dates were selected at their final maturity stage ("*tamar*" stage) and in a dry condition. The date seed preparations were performed according to the method described by Besbes *et al.* (2004b). The steps included manual seed removal from the dates before washing to eliminate the remaining date flesh on the seeds, and then dried at 50°C until their moisture content was less than 5%. Then, a high-speed grinder (Hamilton Beach Commercial, Hamilton Beach Brands Inc., USA) was used to grind the seeds into powder. The powdered form of the seeds was then sieved using a tea strainer with a 0.5 mm pore size before stored in sealed containers at room temperature until further processing.

Oil extraction using Soxhlet extraction method

A two-litre Soxhlet apparatus was used to extract the date seed oil (DSO), in which 300 g powdered date seed was utilised in each extraction. Petroleum benzine was used as the solvent, and the extraction was carried out at 60°C for 8 h. The solvent evaporated at 60°C with the use of a rotary evaporator (Model Eyela N-1000, Rikakikai Co. Ltd., Tokyo, Japan). The resulting oil was stored in an amber bottle at 4°C until further analysis.

Determination of Iodine Value

Iodine value (IV) of the DSO was determined according to the PORIM Test Method (1995). briefly, 20 mL cyclohexane was added to 0.3 g DSO to dissolve; following which 25 mL Wij's solution was poured into the oil mixture and gently shaken prior to being stored in the dark for 1 h. Then, 20 mL potassium iodide and 100 mL distilled water were added into the solution and titrated with 0.1 N sodium thiosulphate solution until the yellow colour of iodine almost disappeared. Lastly, 1 mL starch solution was added, and the titration process was continued until the blue colour disappeared. The iodine value was expressed as gram of iodine absorbed by 100 g of oils (g I2/100 g).

Determination of fatty acid composition

The fatty acid composition (FAC) of the DSO was analysed by determining its fatty acid methyl ester (FAME). The FAME was determined by dissolving 50 µL oil into 1,000 µL hexane prior to the addition of 100 µL 1 M sodium methoxide solution. After 10 min, the mixture was centrifuged to ease the collection process of FAME layer from the mixture (PORIM Test Methods, 1995). Then, the resulting FAME was analysed using gas chromatography-flame ionisation detector (GC-FID) (Agilent 6890 N Network GC System, Agilent Technologies, Singapore) for fatty acid determination. A polar capillary column DB-WAX having 0.25 mm internal diameter, 30 m length and 0.25 µm film thickness (J&W Scientific, Agilent Technologies, USA) was used for the analysis. The oven temperature was set up as follows: the initial temperature was set at 120°C for 1 min before being increased to 200°C at 5°C/min for 10 min, followed by 20°C/min increments until the temperature reached 240°C for 6 min. The injector and detector temperatures were kept at 210°C throughout the analyses, whereby helium and hydrogen gases were utilised as the carrier gases in a 50:1 split ratio. Peaks identification was done by comparing the sample's peaks with a chromatographic profile containing FAME standard (Supelco, Bellefonte, PA). Meanwhile, the percentage of FAC was determined based on the partial peak area over total peak area.

Determination of triacylglycerol composition

The triacylglycerol (TAG) composition was analysed using a high-performance liquid chromatography (HPLC; model Waters 2695)

equipped with a refractive index detector (model Waters 2414; Waters Associates Inc., Milford, MA). The process was conducted by utilising a Merck LiChrospher RP-18 column (12.5 cm × 4 mm internal diameter) (Merck, Darmstadt, Germany), based on the method described by Yanty *et al.* (2011). Acetone:acetonitrile (63.5:36.5 v/v) was used as the mobile phase at 1.5 mL/min flow rate, with the injection of 10 µL 5% (w/w) oil sample in chloroform into the HPLC. The oven temperature was maintained at 30°C throughout the analysis. The resulting TAG peaks were then analysed using Empower software (Milford, MA) and presented as percentage areas, whereby their identification were done according to the TAG profiles of DSO reported previously by Nehdi *et al.* (2010).

Determination of thermal profile

The thermal analysis was performed using differential scanning calorimetry (DSC) 823^e fitted with a sample robot (Julabo FT400 Intracooler, Julabo, Germany), and thermal analysis data station (STARe Software, Version 9.0x, Schwerzenbach, Switzerland) for data analysis. Nitrogen gas of 99.99% purity was used as the purge gas used throughout the analysis, with a flow rate of ± 20 mL/min.

Approximately 6 mg of each oil sample was weighed into an aluminium pan and hermetically sealed, with an empty sealed aluminium pan being used as a control and reference. The analysis was subsequently performed as follows: the sample was held at 70°C for 1 min to eliminate the thermal history of the sealed samples before the temperature was decreased to -90°C at 5°C/min, and then held for another 1 min. Next, it was increased to 70°C at a rate of 5°C/min (Marikkar *et al.*, 2003).

Determination of total phenolic content

The total phenolic content was determined based on the method described by Folin and Ciocalteu (1927), Singleton and Rossi (1965), and Miliauskas *et al.* (2004), with slight modifications. The oils were diluted to 0.5 mg/mL in dimethyl sulfoxide (DMSO), followed by 0.5 mL of the diluted oils being mixed with 2.5 mL of 10% Folin-Ciocalteu reagent. After the mixture was vortexed for 10 s, 2 mL 7.5% sodium carbonate was added and subsequently vortexed for another 10 s. Then, the mixtures were incubated at 40°C for 1 h, whereby the resulting mixture absorbance was measured using microplate reader (Infinite M200, TECAN, Austria) at 765 nm. The measurement was done with deionised water and gallic acid served as the blank and standard, respectively. The curve was then prepared at the modified concentrations of 0.1, 0.05, 0.025, 0.0125 and 0.00625 mg/mL. Finally, the total phenolic content was expressed as the gallic acid equivalent (GAE) per 1 g of oil, which was calculated using the following equation:

$$C = \frac{c}{m} \times V$$

where C = total content of phenolic compounds of extracts (mg/g), or mentioned as GAE; c = concentration of gallic acid (mg/mL) from the calibration curve; V = volume of oil (mL); and m = weight of plant crude extract (g).

A standard curve was prepared from the five concentrations of gallic acid in DMSO at 6.25, 12.5, 25, 50 and 100 ppm respectively. The R^2 value derived from the plotted absorbance was 0.9998. The fitting equation, $y = 0.007x + 0.064$ obtained from the standard curve was then used in determining the GAE value of the DSOs.

Determination of radical scavenging activity using DPPH

The free radical scavenging activity was determined using DPPH based on the method described by Moein *et al.* (2007), with slight modifications. The oils were prepared at different modified concentrations of 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/mL in DMSO. Next, 200 µL of modified 0.025 mg/mL methanolic DPPH was added into 20 µL of each oil dilution, and kept in the dark at room temperature for 30 min. The resulting absorbance was determined using microplate reader at 517 nm against DMSO as the blank and gallic acid as the standard. The antioxidant activity (AOA) was calculated by using the following equation:

$$\mathrm{AOA} = 100 - \left(\frac{\alpha_s - \alpha_b}{\alpha_c} \times 100\right)$$

where α_s = sample absorbance; α_b = blank absorbance; and α_c = control absorbance.

Determination of reducing power using FRAP method

Spectrophotometric FRAP method was performed according to the method described by Benzie and Strain (1996) and Szydlowska-Czerniak *et al.* (2008), with slight modifications. Briefly, the FRAP reagent was first prepared from the respective mixtures of 2.5 mL 10 mM TPTZ solution in 40 mM HCl, 2.5 mL 20

mM $FeCl_3$, and 25 mL 0.1 M acetate buffer (pH 3.6) made up of acetic acid and sodium acetate. They were then incubated at 37°C for 10 min. Next, 1 mL of oil of different concentrations in DMSO (i.e. 0, 0.25, 0.5, 1.0, 2.0 and 4.0 mg/mL) was added to 2 mL FRAP reagent, and brought to 10 mL with redistilled water. After being left at room temperature for 10 min, their respective absorbance was measured against the blank at 593 nm using the microplate reader. Reagent blank was specifically prepared using a mixture of 2 mL FRAP reagent and addition of redistilled water to reach 10 mL. Meanwhile, the gallic acid calibration curve was prepared by dissolving gallic acid in DMSO at the modified concentrations of 0.25, 0.5, 1.0, 2.0 and 4.0 mg/mL.

Statistical analysis

All analyses were determined in duplicate measurement unless otherwise stated. The data were analysed with one-way Analysis of Variance (ANOVA) using MINITAB ver. 17 (Minitab Inc., State College, PA, USA) at 0.05 probability level.

Results and discussion

Total oil content and iodine value of date seed oils

The total oil content and IV of five varieties of DSOs are shown in Table 1. The lowest oil content was found to be 8% for Mariami variety, whereas the highest oil content reported was 9.8% for Rotab variety. The oil content of the five varieties ranged between 5.7 and 12.7%, which are similar to reports in previous studies (Besbes *et al.*, 2004a; Besbes *et al.*, 2005; Rahman *et al.*, 2007; Habib and Ibrahim, 2009; Nehdi *et al.*, 2010). In contrast, the oil content of the Safawi, Sufri and Mabroum varieties (8.3, 9.2 and 8.2%, respectively) obtained in the present work were higher than those of Safawi, Sufri and Mabroum varieties (5.9, 7.9 and 6.3%, respectively) obtained from another study (Al-Shahib and Marshall, 2003b). Therefore, this indicated that the same variety of date may have different amount of oil in their seeds, which may be due to the influences of their origin, level of maturation and harvesting time, and the oil extraction procedure itself. According to Al-Farsi and Lee (2008), dates of different maturity stages have different compositions. Thus, the final condition of the fruits is reliant on the parameters during the drying method, such as time length, temperature and humidity. These parameters will also affect the drying process for fruits obtained from different harvesting times due to the inconsistency of the drying method. This henceforth affects the final condition of the fruits and seeds, including its oil content.

Measurement of IV is indicative of the degree of unsaturated fatty acid present in the oil, in which higher IV can be translated to a higher degree of unsaturated fatty acids contained in the oil. In the present work, IVs of DSOs were found to range from 48.7 to 55.7 g I_2/100 g, with Rotab seed oil yielding the lowest IV and Safawi seed oil the highest. The IV obtained in the present work were found to be lower than those yielded by Nehdi *et al.* (2010), Boukouada *et al.* (2014) and Al Juhaimi *et al.* (2018). Such difference may be attributed to the different types of palm species utilised, specifically

Table 1. Total oil content, iodine value (IV) and fatty acid composition of date seed oils.

Analysis	Variety of date seed oil				
	Mabroum	Mariami	Rotab	Safawi	Sufri
Oil cont. (%)	8.16 ± 0.01^{d}	8.04 ± 0.01^{e}	9.75 ± 0.04^{a}	8.27 ± 0.00^{c}	9.16 ± 0.03^{b}
IV (g I_2 / 100 g)	50.75 ± 1.63^{b}	52.50 ± 0.00^{ab}	48.70 ± 0.57^{b}	55.65 ± 0.07^{a}	51.85 ± 0.21^{ab}
Fatty acid					
C8:0	nd	nd	0.27 ± 0.0^{a}	nd	nd
C10:0	nd	nd	0.34 ± 0.01^{a}	nd	nd
C12:0	51.53 ± 0.06^{a}	23.18 ± 0.52^{c}	19.18 ± 0.19^{c}	28.03 ± 0.06^{bc}	34.83 ± 3.85^{b}
C14:0	1.18 ± 0.08^{d}	12.29 ± 0.16^{a}	11.70 ± 0.10^{a}	5.55 ± 0.21^{c}	8.17 ± 0.48^{b}
C15:0	0.87 ± 0.16^{a}	nd	nd	nd	nd
C16:0	1.49 ± 0.11^{d}	9.63 ± 0.30^{b}	10.61 ± 0.04^{a}	2.96 ± 0.09^{c}	3.36 ± 0.38^{c}
C18:0	4.35 ± 0.62^{c}	6.01 ± 0.07^{b}	7.22 ± 0.03^{a}	3.23 ± 0.01^{d}	1.21 ± 0.02^{e}
C18:1	36.96 ± 1.12^{d}	41.52 ± 0.30^{c}	46.29 ± 0.26^{bc}	59.49 ± 0.12^{a}	51.95 ± 4.01^{b}
C18:2	3.64 ± 0.44^{a}	3.09 ± 0.04^{a}	3.46 ± 0.03^{a}	0.77 ± 0.09^{b}	0.49 ± 0.09^{c}
C18:3	nd	4.29 ± 0.33^{a}	nd	nd	nd
C20:0	nd	nd	0.43 ± 0.01^{a}	nd	nd
C20:1	nd	nd	0.51 ± 0.01^{a}	nd	nd

Values are means ± standard deviation. Means with different superscript letter in the same rows are significantly different at $p < 0.05$. nd: not detected.

Phoenix dactylifera and *P. canariensis*, respectively. Furthermore, the palm plant origin may also cause the dissimilarity, as explained in detail by Boukouada *et al.* (2014) and further relating to the oil's fatty acid composition. Low IV of these DSOs is subsequently indicative of the high content of saturated fatty acids.

Fatty acid composition of date seed oils

Table 1 lists the FAC of all five DSOs, in which eight types of saturated fatty acids and four types of unsaturated fatty acids were found. The findings showed that the different varieties of DSO each having varying fatty acid compositions, with two types of fatty acids dominating, namely oleic acid (C18:1) and lauric acid (C12:0). The major fatty acid in Mariami, Rotab, Safawi and Sufri seed oils were oleic acid, with the respective composition of 41.5, 46.3, 59.5 and 52%. This is followed by lauric acid with the composition of 23.2, 19.2, 28 and 34.8%, respectively. The high value of oleic acid in these four DSOs paralleled with that reported in previous investigations (Al-Showiman, 1990; Devshony *et al.*, 1992; Al-Hooti *et al.*, 1998; Al-Shahib and Marshall, 2003b; Besbes *et al.*, 2004a; Al Juhaimi *et al.*, 2012; Boukouada *et al.*, 2014; Al Juhaimi *et al.*, 2018).

In contrast, Mabroum seed oil contained higher amount of lauric acid (51.5%) than oleic acid (37%), which is comparable with lauric acid content in virgin coconut oil (46.4 to 48.4%), coconut oil (44.1 to 51.3%), and palm kernel oil (44.5 to 52%) (Cornelius, 1977; Mansor *et al.*, 2012). The high amount of lauric acid in virgin coconut oil and coconut oil respectively is linked with monolaurin, which is a monoglyceride derived from lauric acid. The component can act as an antibacterial and antiviral agent (Kabara, 1984; Wang *et al.*, 1993; Enig, 1998). Additionally, Dayrit (2000) stated that the consumption of the specific oil will result in its conversion from lauric acid into monolaurin via bodily systemic breakdown system.

Meanwhile, other fatty acids present in the five DSOs included myristic acid (C14:0), palmitic acid (C16:0), stearic acid (18:0) and linoleic acid (C18:2). Myristic and palmitic acids were found in the Mariami seed oil at 12.3 and 9.6%, respectively, and in Rotab seed oil at 11.7 and 10.6%, respectively. However, several types of fatty acids were only found in certain DSOs, with caprylic acid (C8:0), capric acid (10:0), arachidic acid (C20:0) and gadoleic acid (C20:1) being found only in Rotab seed oil at less than 1% level for each. Moreover, pentadecanoic acid (C15:0) was only yielded from Mabroum seed oil at 0.9%, while linolenic acid (C18:3) was only obtained in Mariami seed oil at 4.3%. Despite the present work having obtained several fatty acids that are only found in certain DSOs, Al Juhaimi *et al.* (2012) found every single fatty acid in each of their DSOs.

Besides, different FAC of the DSOs resulted in different ratios of total saturated fatty acid (SAFA) to unsaturated fatty acid (USFA). SAFA was found to be dominant in Mabroum and Mariami seed oils at 59% and 51%, respectively, whereas Rotab seed oil revealed a 50:50 ratio of SAFA to USFA. Oils with high SAFA content are linked to better storability and prolonged rancidity effect, thus suggesting that these particular types of oil have the potential in making products requiring 'hard' oil (SAFA) as its ingredient. These include margarine, shortening, cocoa butter substitute/equivalent/replacer, creamer, and other similar products. This is an especially beneficial alternative to the hydrogenation process, which typically results in trans fatty acid.

Triacylglycerol composition of date seed oils

The distribution of triacylglycerol (TAG) based on equivalent carbon number (ECN) is presented in Table 2. From the analysis, 28 species of TAG were found in DSOs, with the ECN ranging from ECN 36 to 50. In general, TAG with ECN 44 was underlined as the most dominant species found in the oils, followed by TAG with ECN 42, 46, 48, 40, 50, 38, 36 and 43.

The present work revealed that LaOO was the dominant TAG in the DSOs, which ranged between 15.5 and 18.4%. In contrast, LaOPd and SSCl were only detected in Mabroum and Rotab seed oil, respectively, whereby the presence of these two TAGs in both oils can be attributed to their FAC. In another study, Nehdi *et al.* (2010) found LaLaP + LaMM as the two dominant TAGs in their *P. canariensis* oil. Therefore, the highest tri-unsaturated and tri-saturated TAGs in the five DSOs in the present work were obtained by OOO and LaLaP + LaMM, respectively, which is comparable to the DSO studied by Nehdi *et al.* (2010). However, the report by Abdul Afiq *et al.* (2013) highlighted the lack of study conducted to determine the triacylglycerol composition of *P. dactylifera* seed oil.

Thermal profile of date seed oils

Figure 1 illustrates the thermal behaviour of DSOs. The cooling thermogram in Figure 1 (A) shows that two cooling peaks were obtained from Safawi, Mariami, Sufri and Mabroum seed oils, while Rotab seed oil yielded four cooling peaks. Each peak represents the exothermic energy in terms of heat released during the cooling process and also indicates the number of phase transition that takes place during the solidification process of DSOs. From the curves,

Table 2. Triacylglycerol (TAG) composition of date seed oils.

TAG	ECN	Variety of date seed oil (%)				
		Mabroum	Mariami	Rotab	Safawi	Sufri
LaLaLa	36	3.95	0.87	1.7	0.76	0.92
LaLaM	38	4.24	5.29	2.43	4.29	3.17
LaLaO	40	1.54	2.15	1.97	1.98	1.81
LaLaP + LaMM	40	10.96	9.88	10.83	8.19	10.86
LaMO	42	3.23	2.15	1.64	1.77	2.01
LaMP	42	4.91	5.99	3.76	5.8	5.56
MLL + LLL + LaLO	42	13.12	9.48	11.77	11.92	13.36
LaOPd	43	3.28	nd	nd	nd	nd
SSCl	44	nd	nd	1.19	nd	nd
LaOO	44	16.52	16.6	15.54	18.44	17.39
LaPO + POLn	44	11.29	11.63	11.73	9.47	10.3
OOL	46	2.33	6.21	2.33	4.33	2.1
MOO	46	4.31	8.84	9.24	8.96	10.91
LaSO + SSCp + SOLn + POL	46	4.75	5.74	6.95	5.67	6.38
OOO	48	4.07	8.01	4.81	8.08	5.62
POO + PGL + ALL	48	3.11	4.49	7.04	5.79	3.89
POP	48	2.11	1.51	3.66	2.35	2.32
SOO + POS	50	6.3	1.16	3.04	2.19	3.37

ECN: equivalent carbon number; nd: not detected; Cl: caprylic acid; Cp: capric acid; La: lauric acid; M: myristic acid; Pd: pentadecanoic acid; P: palmitic acid; S: stearic acid; O: oleic acid; L: linoleic acid; Ln: linolenic acid; A: arachidic acid; G: Gadoleic acid.

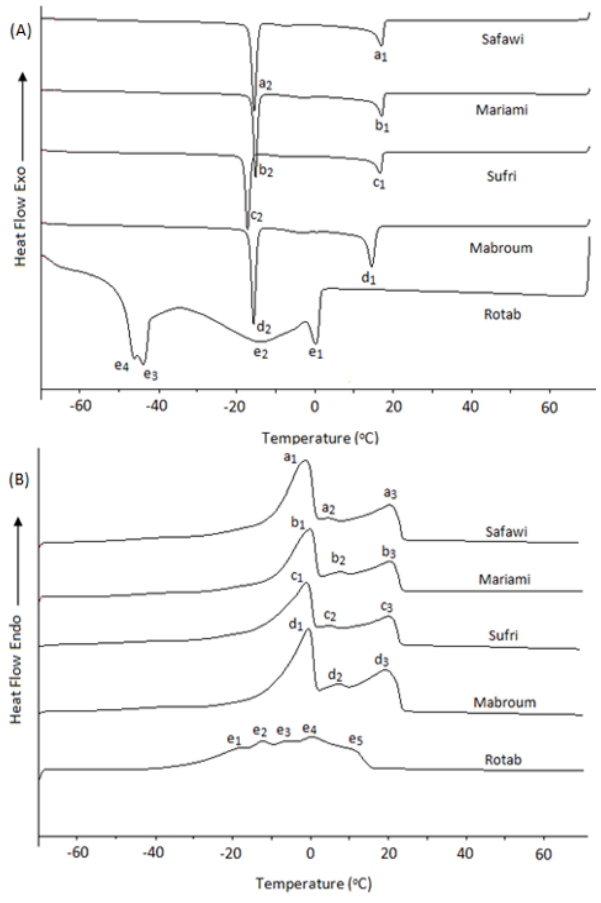

Figure 1. Cooling (A) and heating (B) thermograms of date seed oils

the solidification process for Safawi, Mariami, Sufri, Mabroum and Rotab seed oils started at 17.9°C (a_1), 17.8°C (b_1), 17.4°C (c_1), 15.9°C (d_1) and 1.53°C (e_1), respectively, and stopped at -16.6°C (a_2), -16.1°C (b_2), -18.2°C (c_2), -16.7°C (d_2) and -46.5°C (e_4), respectively. Similar trends were seen for the cooling process of all varieties of DSO, except for Rotab. Despite Rotab seed oil having second solidification curve (e_2, -14.5°C) that was comparable to other DSOs, the solidification process of this particular oil did not cease at this point. It continued to form e_3 (-43.5°C) curve and indicated that the phase transition was still on-going, before stopping only at e_4 (-46.6°C) curve. This was not obtained by the other DSOs.

In the heating thermogram shown in Figure 1 (B), three melting peaks were obtained from Safawi, Mariami, Sufri and Mabroum seed oils respectively. Each melting peak represents the phase transition of DSOs that takes place during the heating process. In contrast with the cooling thermogram, each melting peak specifies the endothermic energy in terms of heat absorbed by the oils during the heating process. The onset temperature of the melting process for Safawi, Mariami, Sufri, Mabroum and Rotab seed oils started at -9.5°C (a_1), -8.5°C (b_1), -8.9°C (c_1), -8.6°C (d_1) and -22°C (e_1), respectively, while the endset temperature were recorded at 23.5°C (a_3), 23.2°C (b_3), 22.9°C (c_3),

23.4°C (d_3) and 16.3°C (e_5), respectively. The Rotab seed oil revealed different thermal behaviour again with five melting and cooling curves obtained in the process, thus indicating the different phase transition for the oil. The temperatures of the second, third and fourth melting curves of Rotab seed oil are -12.6°C (e_2), -7.38°C (e_3) and -0.39°C (e_4), respectively.

The difference in the thermal profile for Rotab seed oil as compared to the other DSOs may be due to the variation of the FAC and TAG of the oil as stated by Besbes *et al.* (2005), Nehdi *et al.* (2010), and Yanty *et al.* (2011). The presence of short chain fatty acids, namely caprylic (C8:0) and capric (C10:0) acids, as well as long chain fatty acids, namely arachidic (C20:0) and gadoleic (C20:1) acids in the seed oil, which were not detected in other DSOs influenced its solidification and melting process. This can be seen in the cooling and heating thermograms obtaineda in the present work. The presence of saturated long chain fatty acids in the seed oil will explain the later initiation of solidification in the cooling as process compared to the other DSOs that contained saturated medium chain fatty acids. In contrast, the presence of short chain fatty acids will ease the melting of oil that took place during the heating process.

The identification of thermal profile has been used to determine oil quality, as evidenced by Marina's (2009) work in the authentication and adulteration detection of virgin coconut oil. For DSO, Nehdi *et al.* (2010) and Besbes *et al.* (2004a) found that the onset temperature for the melting of their oils to be -13.6°C and -19.0 to -21.7°C, respectively, which is comparable to DSOs obtained in the present work.

Total phenolic content

The total phenolic content obtained from the DSOs ranged from 7.962 to 17.724 mg/g as shown in Table 3. The highest phenolic content was found in Safawi seed oil (17.724 mg/g), followed by Sufri seed oil (17.333 mg/g), Mariami seed oil (15.124 mg/g), Mabroum seed oil (12.181 mg/g) and Rotab seed oil (7.962 mg/g). The results substantiated that different varieties of DSOs contain varying amounts of phenolic content, in which such difference may be caused by variations of the fruit's origin, cultivation region, and cultivars of the date fruits. These elements may subsequently influence the nutritional characteristics of the fruits and seeds.

Besbes *et al.* (2004c) found that the total phenolic content of two DSOs, namely Deglet Nour and Allig to be 520.81 and 220.32 mg/kg, respectively. Meanwhile, Boukouada *et al.* (2014) reported the total phenolic content of five DSOs to be ranging between 0.64 and 1.27 mg/g, whereas Al Juhaimi *et al.* (2012) reported that the total phenolic content of seven date seed extracts ranged between 1.98 and 4.95 mg GAE/100 g. Another study by Bilgari *et al.* (2008) also highlighted higher total phenolic content of dry date as compared to the soft and semi-dry date. This shows that the conditions of the date itself can be linked to its resulting total phenolic content. As the date seed is located at the centre and inside the fruit, the dryness of the date may also affect the dryness of the seed, thus affecting its total phenolic content.

The total phenolic content of DSOs obtained in the present work is in agreement with Besbes *et al.* (2004c), as the DSOs have higher values as compared to olive oil (averaged at 500 mg/kg) (Tuck and Hayball, 2002) and virgin coconut oil (12 - 25 mg/100 g) (Marina *et al.*, 2009). According to Nevin and Rajamohan (2004), virgin coconut oil may emit beneficial effects to the body system due to its phenolic content, such as reduced total cholesterols, triacylglycerols, phospholipids, low density lipoprotein (LDL) and very low density lipoprotein (VLDL) levels, as well as elevated high

Table 3. Antioxidant activity at different concentrations, free radical scavenging activity in terms of EC_{50}, and total phenolic content of date seed oils and gallic acid.

Oil variety	Antioxidant Activity (AOA) (%)						
	Concentration (mg/mL)					EC_{50} (mg/mL)	Total phenolic content (mg GAE/g oil)
	0.25	0.5	1.0	2.0	4.0		
Sufri	35.32 ± 1.35^{bB}	36.67 ± 0.37^{bB}	36.84 ± 1.10^{bB}	41.41 ± 0.09^{bA}	41.97 ± 1.32^{bA}	7.965	17.333 ± 0.709^{a}
Safawi	32.21 ± 0.23^{bC}	33.90 ± 0.55^{bB}	38.87 ± 1.59^{bB}	40.82 ± 0.98^{bAB}	45.22 ± 2.48^{bA}	5.170	17.724 ± 0.635^{a}
Mariami	34.46 ± 0.31^{bA}	36.69 ± 2.66^{bA}	37.62 ± 0.25^{bA}	37.92 ± 0.92^{bA}	38.70 ± 1.16^{bA}	16.832	15.124 ± 0.683^{a}
Rotab	35.11 ± 0.61^{bA}	35.65 ± 0.52^{bA}	37.32 ± 2.63^{bA}	37.92 ± 1.47^{bA}	38.51 ± 0.21^{bA}	17.175	7.962 ± 0.483^{c}
Mabroum	36.67 ± 1.41^{abA}	37.34 ± 1.44^{bA}	39.22 ± 0.74^{bA}	39.87 ± 2.08^{bA}	40.69 ± 0.18^{bA}	12.962	12.181 ± 0.886^{b}
Gallic acid	40.65 ± 1.84^{aC}	65.28 ± 1.84^{aB}	97.64 ± 0.03^{aA}	98.44 ± 0.31^{aA}	97.97 ± 0.00^{aA}	1.004	-

Values are means ± standard deviation. Means with different superscript lower-case and upper-case letter in the same columns and rows, respectively, are significantly different ($p < 0.05$).

density lipoprotein (HDL) in serum and tissues. The combination of low LDL and VLDL level with high HDL level in the blood circulation may lower the risk of arteriosclerosis and coronary heart disease. However, further study needs to be conducted to substantiate the relationship between phenolic content in DSO with the beneficial effects to the body system.

Determination of radical scavenging activity using DPPH

The determination of radical scavenging activity was done by analysing the capability of phenolic compound present in the seed oil to act as hydrogen or electron donor and subsequently deactivating free radical, using 1,1-diphenyl-2-picrylhydrazyl (DPPH) reagent. In this analysis, the reduction of the violet colour of DPPH to yellow colour indicated the presence of antioxidants in the oil. Furthermore, the low intensity of the yellow colour is synonymous with donation or pairing of more hydrogen or electrons with DPPH radicals, thus suggesting the high content of antioxidant compounds present in the oil.

Table 3 shows the percentage of antioxidant activity of DSOs and gallic acid at 0.25, 0.5, 1, 2 and 4 mg/mL. It is clear from the Table that when the concentration increased from 0.25 to 4 mg/mL, the highest increment (13%) of antioxidant activity in DSO was obtained by the Safawi seed oil. Meanwhile, the lowest increment (3.4%) was seen in Rotab seed oil. At 4 mg/mL, the highest radical scavenging activity was yielded by Safawi seed oil, indicating its better capability in donating hydrogen or electron from its phenolic compounds in comparison with the remaining four DSOs. In contrast, the highest radical scavenging activity was obtained by Mabroum seed oil (36.7, 37.3 and 39.2%, respectively) at the concentrations of 0.25, 0.5 and 1 mg/mL, respectively, whereas 2 mg/mL yielded the highest radical scavenging activity by Sufri seed oil (41.4%).

Furthermore, the result also depicted a significant difference in antioxidant activity between DSOs and gallic acid. The antioxidant activity of gallic acid was found to increase when the concentration increased from 0.25 to 1 mg/mL. However, further increment from 1 to 4 mg/mL resulted in no significant difference to such activity, which indicated that the maximum antioxidant activity of gallic acid was achieved at 1 mg/mL. In contrast, Al Juhaimi *et al.* (2012) reported that the antioxidant activity from date seed extracts ranged between 78 and 80 mg/mL.

In the present work, the efficiency concentration (EC_{50}) to reduce 50% of DPPH radicals was also determined, with Table 3 displaying the results of EC_{50} which indicate the concentration of DSOs and gallic acid needed to achieve it. The low EC_{50} value was indicative of the high radical scavenging activity of the sample. Table 3 also displays the EC_{50} value obtained in the order of gallic acid < Safawi seed oil < Sufri seed oil < Mabroum seed oil < Mariami seed oil < Rotab seed oil. From the five DSOs, Safawi yielded the highest antioxidant activity (5.170 mg/mL), while Rotab the lowest (17.175 mg/mL). Nevertheless, the EC_{50} in the present work was lower than those reported by Boukouada *et al.* (2014). Moure *et al.* (2001) and Marina *et al.* (2009) substantiated the different radical scavenging activity of the oil to be due to the processing conditions. Furthermore, the radical scavenging activity of virgin coconut oil obtained by Marina *et al.* (2009) in terms of EC_{50} value was below 4 mg/mL, suggesting its higher antioxidant activity as compared to DSOs in the present work. However, the EC_{50} values of DSOs obtained in the present work were higher than those of extra virgin olive oil, olive oil, soybean oil, sunflower oil and corn oil (Valavanidis *et al.*, 2004).

Besbes *et al.* (2004c) identified eight types of phenolic compounds in their DSOs, whereby more than 50% of the total phenolic compounds could not be identified. Therefore, they concluded that DSO is better than olive oil in terms of phenolic content. Al Juhaimi *et al.* (2018) found five predominant phenolic compounds in date seed oil, whilst Özcan and Al Juhaimi (2015) reported that a better shelf life of olive oil was obtained with the addition of date seed extract into the oil. Table 3 also shows that the highest total phenolic content obtained by Safawi seed oil is in agreement with the analysis of antioxidant activity and EC_{50} value, accordingly. This is indicative of the effectiveness of phenolic content in this particular oil to act as an electron or hydrogen donor towards inhibiting oxidation of free radicals. The same trend was also shown by Rotab seed oil, which yielded the lowest total phenolic content, antioxidant activity and EC_{50} value, respectively.

Measurement of reducing power using FRAP method

Figure 2 illustrates the reducing power capability of DSOs. The reducing capability of DSOs was not significantly increased at the concentrations of 0 to 1 mg/mL. However, increments of reducing power capability of the oils were observed at 2 mg/mL concentration and continued to increase significantly as the concentration further increased to 4 mg/mL. This indicates that concentration below than 1 mg/mL bears no significant effect toward their

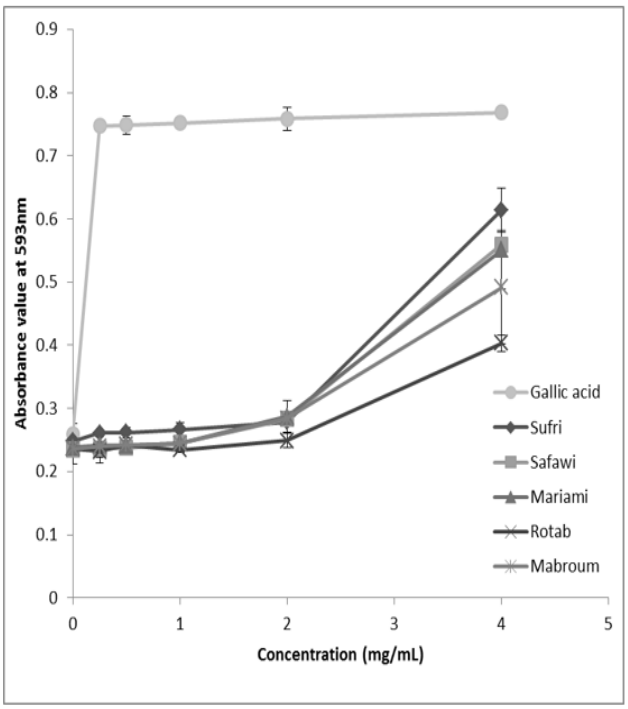

Figure 2. Reducing power of date seed oils and gallic acid in terms of absorbance value at 593 nm.

capability to reduce Fe^{3+} ions. Regardless, Sufri seed oil yielded the highest reducing power capability (0.6139AU) from the five DSOs, followed by Safawi seed oil (0.5591AU), Mariami seed oil (0.5512AU), Mabroum seed oil (0.4918AU), and Rotab seed oil (0.403AU).

In contrast, a different trend of reducing power capability was shown by gallic acid that acted as a standard to the analysis. It reached its maximum reducing power capability at the concentrations between 0 to 0.25 mg/mL. However, the graph allowed the prediction that further increase in the concentration of DSOs will further elevate the absorbance reading, thus increasing their reducing power capability. This is as shown by the increasing graph obtained at 4 mg/mL thereby suggesting the possibility of getting the same reducing power capability as shown by gallic acid.

Conclusion

The present work found a good range of oil content in the five varieties of date seeds, as well as additional benefits due to their physicochemical and antioxidant properties. Different characteristics displayed from the date seed oils suggest the potential for various applications to be developed for the oils. However, further study may be needed to determine the safety of these oils either for consumption or other uses to substantiate the worth behind extracting these oils in the future.

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