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FULL LENGTH ARTICLE

Phytochemical compositions and antioxidant capacity of three date (*Phoenix dactylifera* L.) seeds varieties grown in the South East Morocco

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Abstract Three Moroccan date seeds (*Phoenix dactylifera* L.) varieties (*Majhoul*, *Boufgous* and *Bousthammi*) were evaluated for their proximate, phytochemical and nutrient compositions. The crude fiber ranges between 15.84–19.9 g/100 g DW, moisture (4.554–8.259%), protein (4.309–6.144% of DW), ash (1.097–1.3% DW) and fat (5.662–6.972% DW). The most abundant fatty acids of date seed oils as revealed gas chromatography were oleic, lauric, myristic, palmitic and linoleic acids. The physicochemical analysis of date seeds oil shows an acid value between 1.083–1.813 mg KOH/g, saponification value (202.33–222.74 mg KOH/g), peroxide value (1.243–1.01 meq O_2/kg) and iodine value (45.40 and 58.02 g Iodine/100 g). The unsaponifiable matter of date seed oils was found between 0.62% and 1.103%. Among the eight studied minerals potassium, magnesium and calcium were the predominant of macroelement and iron was the predominance of microelement. The antioxidant of date seeds assessed using three assays varied between 10.966–22.86 mmol Trolox equivalent/100 g DW, 4.807–8.021 mmol Trolox equivalent/100 g DW and 0.166–0.112 g/l for FRAP, ABTS and IC50 of DPPH respectively. The phenolic and the flavonoid content of date seeds found changed between 2697–5342 mg Gallic acid equivalent/100 g DW and 1224–1844 mg Rutin equivalent/100 g DW respectively. Results showed that date seeds could be used as ingredients to enhance the nutritional value of some functional

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foods for human consumption as well as using additives in food, pharmaceutical and cosmetic industries.

1. Introduction

The fruit of the date palm (*Phoenix dactylifera* L.) is one of the important agricultural commodities in the Moroccan Sahara. They are served mainly as a vital component of the diet, staple food and constitute the principal source of remuneration and the basis of economy for the people of these regions. Date seeds represent a major waste material and constitute approximately 6.10–11.47% of the fruit (Habib and Ibrahim, 2009). Date seeds can be discarded, used in animal feeding or used in making non-caffeinated coffee (Habib and Ibrahim, 2009). They are also listed in folk remedies for the management of diabetes, liver diseases and gastrointestinal disorders in traditional Egyptian medicine (Duke, 1992). It has been reported that the extracts of date seeds ameliorate gastric ulceration in rats (Al Qarawi et al., 2005) and possess an anti-inflammatory activity in the rat adjuvant arthritis model (Doha and Al-Okbi, 2004). Salah and Al-Maiman (2005) have reported that feeding the defatted date seed flour to rats reduced the plasma triglycerides, total cholesterol and low-density lipoprotein.

The date fruit seeds contain a wide range of nutritional functional compounds such as fiber, fat, moisture, protein, ash and vitamins as well as high amounts of phenolic (Al-Farsi et al., 2007).

The objectives of this research were evaluated proximate, phytochemical and nutrient compositions of three Moroccan date seeds (*P. dactylifera* L.) Varieties include protein, crude fiber, moisture, ash, minerals, and fat and analyzed the composition on fatty acids, as well as phenolic and flavonoid content and evaluate the antioxidant activities.

2. Materials and methods

2.1. Materials

Date fruit varieties were obtained at Tamr stage from Errachidia National Institute for Agricultural Research.

Chemicals and reagents: The compounds 2,2-diphenyl-1-picryhydrazyl radical (DPPH), 2,2′-azinobis (3-ethylbenzothiazoline-6-sulfonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-S-triazine (TPTZ), 6-hydroxy-2,5,7,8-tetramethyl chroman-2-carboxylic acid (Trolox), Gallic acid, Rutin, potassium persulfate and fatty acid methyl gas chromatography (GC) standards were acquired from Sigma–Aldrich (Dorset, UK). Folin–Ciocalteu reagent, sodium acetate, and sodium carbonate, sodium hydroxide, sodium nitrite, $FeCl_3{\cdot}3H_2O$, hydrochloric acid, phenol, sulfuric acid and methanol, hexane were from Merck (Germany).

Instruments: Gas chromatograph (Perkin Elmer, Clarus 580, USA), atomic absorption spectrometer (Perkin Elmer, AAnalyst 200 Model, USA), Spectrophotometer (RAYLEIGH, VIS-723G, china), Pulverisette 15 cutting mill (Fritsch, Germany).

2.2. Physical properties of date seeds

Ten date fruits were taken randomly from each variety to determine seed/date fruit weight ratio and dimensions (length and diameter).

2.3. Preparation of date seed powder

The seeds were directly isolated from three date fruits named locally *Bousthammi*, *Majhoul* and *Boufgous*. The seeds of each variety were separately washed, dried and grounded into a fine powder using Cutting Mill.

2.4. Proximate composition of date seeds

Total nitrogen was determined by the Kjeldahl method (AOAC, 1997) and then the protein amount was calculated using a factor of 6.25. The moisture was determined by oven-drying at 105 °C to constant weight (AOAC, 1997). Total sugar content was determined using the method of Dubois et al. (1956). Crude fiber was determined using AFNOR NF-V 03-040, 1977.

2.5. Determination of energy value

The energy values of dates, varieties were evaluated using the formula described by Crisan and Sands (1978).

$$\text{Energy value(kcal/100 g)} = (2.62 \times \% \text{ protein}) + (8.37 \times \% \text{ fat}) + (4.2 \times \% \text{ carbohydrate})$$

2.6. Extraction of date seed fats

The seed lipids of each variety were extracted in a soxhlet apparatus using n-Hexane as a solvent for 8 h. The solvent was removed using a rotary evaporator at 40 °C and the lipids were weighed and stored in a freezer at 4 °C until analysis.

2.7. Physicochemical characteristics of oils

The acid value, saponification value, peroxide value and unsaponifiable matter of the seed oils were determined according to the AFNOR methods such as AFNOR NFT 60-204, AFNOR. T60-206, AFNOR NFT 60-220 and AFNOR NFT 60-205 respectively. The iodine value was determined using AOCS Cd Id-92.

2.8. Fatty acid analysis

The preparation of fatty acid methyl esters was done using transesterification with methanolic potassium hydroxide. The

method used in this study is described in AFNOR NF EN ISO 5508. The analysis was performed using gas chromatograph equipped with a flame-ionization detector and capillary column (30 m × 320 μm i.d, 0.25 μm film thickness). The injection volume was 1 μL, and the carrier gas was Helium. The Helium and air flows of FID detector were 45 and 300 ml/min, respectively. The oven temperature was 100 °C at 0 min and was raised to 200 °C at a rate of 6 °C/min and hold for 50 min. The injector and the detector were maintained at 260 and 280 °C, respectively. The peaks were recognized, based on their retention times (RT) using standard FAMEs. All samples were run in triplicate.

2.9. Minerals determination

The method of AOAC (1997) was employed for the determination of ash and mineral content. Two grams of the pulverized samples were placed in a crucible, and ignited in a muffle furnace overnight at 550 °C and then cooled in a desiccator and weighed at room temperature to get the weight of the ash. To the resulting ash 5 ml of concentrated chloride acid was added, and evaporated on a hot plate, some drops of H_2O_2 and 5 ml of bidistilled water were added and filtered in 100 ml volumetric flasks and then the volume was made up with bidistilled water.

This solution was used for the determination of mineral content. Atomic absorption spectrophotometer (AAS) was used to determine Mg, Fe, Mn, Cu, Ca, K, Na and Zn.

2.10. Preparation of rich polyphenol extracts

The rich phenolic extract was prepared according to the method of Bouhlali et al. (2017). Briefly, 30 g of date seeds powder was extracted with 150 ml of methanol–water (4:1, v/v), at 35 °C for 12 h using an orbital shaker-incubator. The mixture was then filtered and the filtrate was concentrated under reduced pressure at 40 °C until total evaporation of solvent using a rotary evaporator. The results of methanolic crude extract were kept at −20 °C in dark glass bottles until use.

2.11. Measurement of total phenolic compounds

The total phenolic contents in date seeds were determined according to the method described by the International Organization for Standardization (ISO 14502-1). Briefly, 100 μL of the extract was added to 500 μL of a 1/10 dilution of Folin–Ciocalteu reagent in water, and then 400 μL of sodium carbonate solution (7.5% w/v) was added. The mixture was left for 60 min at room temperature and the absorbance was measured at 765 nm. The calibration curve was prepared using Gallic acid. The total phenolic compounds were expressed as Gallic acid equivalent in mg/100 g dry weight (DW) date seed.

2.12. Measurement of flavonoid content

The total flavonoid content of date was determined by the method of Kim et al. (2003). One ml of date seeds extract was mixed with 4 ml of distilled water. Then 0.3 ml of sodium nitrite solution (5%) was added, followed by 0.3 ml aluminum chloride solution (10%). Test tubes were incubated for 5 min at ambient temperature, then 2 ml of sodium hydroxide (1 M) was added to the mixture and then the final volume was made up to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance was determined at 510 nm. Measurements were calibrated to a standard curve of prepared Rutin solution and the results were expressed as mg Rutin equivalents (RE)/100 of dry weight (DW).

2.13. ABTS radical scavenging assay

The ABTS radical scavenging was measured using the method of Re et al. (1999). The ABTS radical cations (ABTS+) were produced by reacting aqueous solution of ABTS (7 mM) with aqueous solution of potassium persulfate (2.45 mM). The mixture was allowed to stand in the dark at room temperature for 12–16 h before use and then diluted with distilled water to obtain an absorbance of 0.700 ± 0.005 at 734 nm. 30 μL of the sample added to 3 ml of the ABTS radical solution was allowed at room temperature for 6 min and the absorbance at 734 nm was recorded immediately. A standard curve was obtained by using aqueous solution of Trolox. Total antioxidants were expressed as grams of Trolox equivalents per 100 g of dry weight (DW) date seeds.

2.14. Ferric reducing antioxidant power assay

The ferric reducing activity of date seed extract was estimated based on the method of Benzie and Strain (1999). The FRAP reagent was prepared by mixing 50 ml of acetate buffer (0.3 M) at pH 3.6, 5 ml of tripyridyltriazine (TPTZ) solution 10 mM prepared in HCl (40 mM) and 5 ml of ferric chloride solution ($FeCl_3$) (20 mM). 2 ml of the freshly prepared FRAP reagent was added to 10 μL of the extract. Then the absorbance was measured at 593 nm against the blank after 10 min at room temperature. The standard curve was constructed using Trolox. The result was expressed as Trolox equivalent in mg/100 g of dry weight (DW) date seed.

2.15. DPPH radical scavenging activity

Scavenging Radical activity of date seed against stable DPPH was assessed as described by Blois (1958) method with slight modifications. The reaction mixture contained 100 μL of date seed at different concentrations and 1.9 ml of methanolic DPPH (0.3 mM). The mixture was incubated at room temperature for 20 min and the absorbance was determined at 517 nm. The IC50 (concentration providing 50% inhibition) values were calculated from the plotted graph of scavenging activity against the concentrations of the samples.

2.16. Statistical analysis

Statistical analysis was performed using StatView 5.0 software. The experimental results were reported as mean ± SE (standard error) ($n = 6$) on a dry weight. Analysis of variance (ANOVA) and post hoc Bonferroni ($p < 0.0018$) tests were used to compare the experimental groups. Pearson's correlation coefficient (r) was used to measure the association

Table 1 Physical properties of date seeds.

	Boufgous seeds	*Bousthammi* seeds	*Majhoul* seeds
Seed/date fruit mass ratio	9.45 ± 0.22	10.34 ± 0.37	7.29 ± 0.17
Diameter (cm)	0.99 ± 0.07	0.77 ± 0.047	1.15 ± 0.057
Length (cm)	2.58 ± 0.063	1.71 ± 0.046	2.75 ± 0.044

Values in average ($n = 6$) ± SE. Averages, in the same line, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

between two variables. Differences at $p < 0.05$ were considered significant.

3. Results and discussion

3.1. Physical properties of date seeds

The dimensions of seeds show a significant difference ($p \leqslant 0.05$) as illustrated in Table 1. The diameter of date seeds varied between 0.77 cm for *Bousthammi seed* and 1.71 cm for *Majhoul seed* which possessed the highest date seed length 2.75 cm, the lowest 1.71 was observed in *Bousthammi seed,* and our results are in agreement with those reported by Al Juhaimi et al. (2012) who founded that the length and the diameter ranged between 14.11–23.22 mm and 6.85–9.02 mm respectively. The highest seed/date fruit weight ratio was observed in *Majhoul seed* and the lowest was observed in *Bousthammi seed*. This characteristic is very used by the farmers to evaluate the quality of varieties. It can be influenced by climate factors and some cultural practices such as reducing the number of dates fruit bunches per palm trees, in addition to the varieties effect.

3.2. Proximate composition

The proximate composition of analyzed date seeds is summarized in Table 2. The highest contents of protein (6.144 g/100 g DW) and crude fiber (19.9 g/100 g DW) were shown in *Majhoul* seeds, which contain the lowest amount of ash (1.097 g/100 g DW) and lipids (5.66 g/100 g DW), but contain the highest amount of total sugar (9.546 g/100 g DW) and moisture (8.26 g/100 g FW). The lower contents of moisture (4.55 g/100 g FW), total sugar (8.70 g/100 g DW) and crude fiber (15.84 g/100 g DW) were determined in *Bousthammi* seeds, which contain the highest amount of lipid (6.97 g/100 g DW). The lowest contents of protein (4.309 g/100 g DW) were determined in *Boufgous* seeds, which contained the highest amount of ash (1.30 g/100 g DW). Our results of proximate composition are very close to those reported by Al Juhaimi et al. (2012), Basuny and Al-Marzooq (2011), Habib and Ibrahim (2009) and Saafi et al. (2008). The results of the analysis showed significant differences ($p \leqslant 0.05$) among crude fiber, fat content, protein content, moisture, but no significant differences ($p \leqslant 0.05$) shown in ash content.

The energy values of analyzed date seeds varied between 103.58 and 108.3 kcal/100 g DW are very lower than those reported by Al juhaimi et al. (2012) who found that the energy values of analyzed seven date seeds were ranged between 4340 kcal/kg and 4795 kcal/kg. The difference may be due to the method used to determine the energy value. That means that the calorimeter determined the raw energy, which incorporates the energy produced by fibers in addition to the energy produced by protein, lipid and digestible carbohydrate determined in our paper.

3.3. Physicochemical characteristic of date seeds oils

The physicochemical characteristic of date seeds oils is presented in Table 3. The acid value of seeds oil ranges between 1.083 mg KOH/g for *Bousthammi* seed oil and 1.813 mg KOH/g for *Majhoul* seed oil, and our results are very close to those reported by Besbes et al. (2004), Boukouada and Yousfi (2009) for Tunisian and Algerian date seeds oil respectively. These results indicate that *Majhoul seed* oil contains a high amount of free fatty acids than *Bousthammi* seeds oil which has the similar free fatty acids content of olive oil analyzed by Borchani et al. (2010), that means that it could be edible.

The saponification value gives an indication on the nature of the fatty acids, which contains the fat and that depends on the average molecular weight of these fatty acids. The saponification value of our seeds oil ranges between 202.33 and 222.74 mg KOH/g of oil for *Majhoul* and *Bousthammi* seeds oils respectively. The high saponification value of date seeds oils indicated that the fatty acids present in the oils have high number of carbon atoms. This means that date seeds oils also, after hydrogenation, could be substituted for some

Table 2 Proximate composition of date seed.

Component	Composition of date seeds on (g/100 g DW)		
	Boufgous seeds	*Bousthammi* seeds	*Majhoul* seeds
Crude fiber	15.84 ± 0.63	18.01 ± 0.315	19.90 ± 0.53
Protein	4.309 ± 0.189	5.116 ± 0.198	6.144 ± 0.07
Lipid	6.763 ± 0.167[a]	6.972 ± 0.465[a]	5.662 ± 0.281
Ash	1.300 ± 0.080[a]	1.267 ± 0.040[a]	1.097 ± 0.054
Moisture	7.432 ± 0.339[a]	4.554 ± 0.078	8.259 ± 0.273[a]
Total sugar	8.712 ± 0.095	8.700 ± 0.076	9.546 ± 0.106
Energy value (kcal)/100 g DW	104.490	108.300	103.581

Values in average ($n = 6$) ± SE. Averages, in the same line, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

Table 3 Physicochemical characteristic of seed oil.

	Boufgous seeds	*Bousthammi* seeds	*Majhoul* seeds
Acid value (mg KOH/g)	1.69 ± 0.04[a]	1.083 ± 0.055	1.813 ± 0.035[a]
Saponification value (mg KOH/g)	214.78 ± 1.54	222.74 ± 1.10	202.33 ± 1.15
Peroxide value meq O_2/kg	1.01 ± 0.03[a]	1.243 ± 0.068[a]	1.043 ± 0.05[a]
Iodine value (g Iodine/100 g)	45.40 ± 1.06[a]	58.02 ± 2.16	50.343 ± 1.066[a]
Insaponifiable matter	1.103 ± 0.062	0.62 ± 0.03[a]	0.827 ± 0.05[a]

Values in average ($n = 6$) ± SE. Averages, in the same line, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

conventional oils in soap and shampoo industry (Akintayo and Bayer, 2002; Falade et al., 2008).

The high peroxide value of *Bousthammi* seed oil (1.243 meq O_2/kg) than *Boufgous* seed oil (1.01 meq O_2/kg) indicates that *Bousthammi* seed oil is most susceptible to autoxidation. These variations can arise from different factors such as the degree of unsaturation of the fatty acids present in the particular oil, storage, exposure to light, and the content of metals or other compounds that may catalyze the oxidation processes (Choe and Min, 2006). In general, the date seeds oil can be considered as safe for human consumption, because of its low peroxide value that is less than 30 meq peroxide/kg (Gotoh and Wada, 2006).

The iodine value, which gives a measure of the average degree of unsaturation of a lipid, changed between 45.40 and 58.02 g Iodine/100 g for *Boufgous seed* and *Bousthammi* seeds oil respectively. This may be due to the fact that *Boufgous* seed oil contained less unsaturated fatty acids than *Bousthammi seed* oil. Our results are higher than those reported by Besbes et al. (2004) but lower than those of Boukouada and Yousfi (2009).

The unsaponifiable matter of date seeds oils ranging between 0.62% and 1.103% was comparable to the result reported by Besbes et al. (2004) but lower than those reported by Basuny and Al-Marzooq (2011) who found 0.776–0.892% and 1.65% respectively.

3.4. Fatty acid composition of date seeds oil

The results of fatty acid analysis of date seeds, which vary slightly within analyzed date seeds varieties, are given in Table 4 and Fig. 1. Among fifteen fatty acids detected oleic was the predominant (44.92–48.38%) followed by lauric (16.74–20.34%), myristic (10.23–12.28%), palmitic (9.82–10.91%), linoleic (8.3–9.02%) and stearic (2.86–3.73%) which composed together more than 98% of the total oil. Arachidic, gadoleic, behinic, tricosylic and lignoceric were present in low amounts and linoleinic, myristoleic palmitoleic, and margaric, are in negligible amounts. The degree of unsaturation of analyzed date seed oil ranged between 53.98% and 57.23% for *Boufgous* and *Bousthammi seeds oil* respectively. These findings are very close to the results presented by Al juhaimi et al. (2012), Besbes et al. (2004) and Sawaya et al. (1984). *Bousthammi* seed oil shows a lower amount of saturated fatty acid 42.79%, but a higher amount of unsaturated fatty acid

Table 4 Fatty acid composition of date seed oil.

Fatty acids	Fatty acid (%) on seeds oil		
	Boufgous	*Bousthammi*	*Majhoul*
(C12:0) Lauric	16.74 ± 0.51[a]	17.02 ± 0.37[a]	20.34 ± 0.41
(C14:0) Myristic	10.23 ± 0.23	12.28 ± 0.14	11.85 ± 0.11
(C14:1) Myristoleic	0.01 ± 0.01[a]	0.07 ± 0.01	0.03 ± 0.02[a]
(C16:0) Palmitic	10.91 ± 0.17[a]	10.65 ± 0.09[a]	9.82 ± 0.12
(C16:1) Palmitoleic	0.05 ± 0.02[a]	0.07 ± 0.01[a]	0.09 ± 0.03[a]
(C17:0) Margaric	0.04 ± 0.01[a]	0.01 ± 0.02[a]	0.02 ± 0.01[a]
(C18:0) Stearic	3.73 ± 0.12	2.86 ± 0.06[a]	2.96 ± 0.09[a]
(C18:1) Oleic	48.38 ± 0.92[a]	46.29 ± 1.17[ab]	44.92 ± 1.53[b]
(C18:2) Linoleic	8.30 ± 0.08[a]	9.02 ± 0.07	8.47 ± 0.10[a]
(C18:3) Linolenic	0.09 ± 0.01	0.15 ± 0.02	0.21 ± 0.03
(C20:0) Arachidic	0.46 ± 0.02[ab]	0.52 ± 0.05[b]	0.43 ± 0.03[b]
(C20:1) Gadoleic	0.40 ± 0.04[a]	0.37 ± 0.02[a]	0.26 ± 0.04
(C22:0) Behenic	0.27 ± 0.01[a]	0.21 ± 0.06[a]	0.25 ± 0.05[a]
(C23:0) Tricosylic	0.26 ± 0.05[a]	0.27 ± 0.03[a]	0.21 ± 0.07[a]
(C24:0) Lignoceric	0.15 ± 0.02[a]	0.21 ± 0.03[b]	0.17 ± 0.04[ab]
SFA	46.03	42.79	44.03
MUFA	45.3	48.84	46.8
PUFA	8.68	8.39	9.17
P/S	0.188	0.196	0.208

SFA: saturated fatty acid, MFA: monounsaturated fatty acids, PUFA: polyunsaturated fatty acids and.
P/S: polyunsaturated/saturated fatty acids.

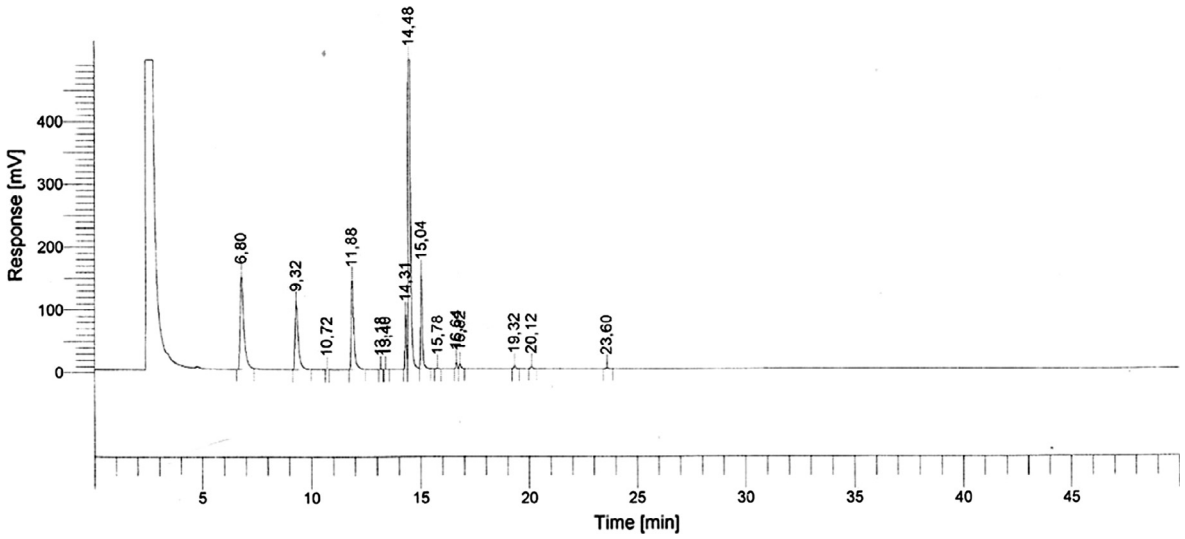

Figure 1 Typical chromatogram of the fatty acids composition of *Bousthammi* seed oil.

Table 5 Mineral composition of date seed varieties.

Minerals	Concentration (mg/kg dry weight)			% RDA mg/day
	Boufgous seeds	*Bousthammi* seeds	*Majhoul* seeds	
Potassium	4153.304 ± 172.484[a]	3355.76 ± 284.448[a]	2967.117 ± 227.680[a]	–
Sodium	128.071 ± 5.078[a]	319.416 ± 17.277	108.060 ± 6.159[a]	–
Magnesium	615.301 ± 8.087	694.676 ± 9.205	827.619 ± 4.983	14.65–26.69%
Calcium	626.71 ± 29.647	490.095 ± 10.637[a]	394.971 ± 8.529[a]	3.94–6.26%
Iron	27.757 ± 2.367	70.298 ± 2.928[a]	55.281 ± 1.291[a]	15.42–87.87%
Copper	4.835 ± 0.318	7.607 ± 0.242[a]	8.358 ± 0.469[a]	53.72–114.65%
Manganese	10.988 ± 0.444	7.679 ± 0.316[a]	5.509 ± 0.361[a]	23.95–61.04%
Zinc	8.768 ± 0.358[a]	10.864 ± 0.955[ab]	14.795 ± 0.065[b]	7.97–18.49%

Values in average ($n = 6$) ± SE. Averages, in the same column, with different letters are significantly different using post hoc Bonferroni tests ($p < 0.001$).

57.21%, which makes it more sensitive to oxidation. The highest amount on saturated fatty acid was shown in *Boufgous* seed oil 46.03%, which contains the lowest amount of unsaturated fatty acid 53.97%. The level of unsaturated fatty acid content of date seed oil was very lower than other vegetable oils. Using three test diets with different PUFA/SFA ratios (P/S ratios 0.27, 1.00 and 1.32), the study by Karupaiah and Sundram (2013) shows that (P/S = 0.27) which is very close to our ratio, was associated with a significantly greater prandial HDL-C trend. In addition, the high amount of lauric acid of these date seeds oils can be decreased significantly TC/HDL-C ratio compared with carbohydrate consumption as shown in the result of Micha and Mozaffarian (2010).

3.5. Mineral contents of date seeds

The content of date seeds on eight minerals that are usually analyzed are given in Table 5. Potassium was the highest mineral content in all samples ranged between 4153.3 mg/kg DW determined in *Boufgous* seeds and 22967.1 mg/kg DW in *Majhoul* seeds, followed by magnesium (827.6–615.3 mg/kg DW) and calcium (626.71–395.0 mg/kg DW). The sodium was the lowest; it varied between 319.4 mg/kg DW in *Bousthammi* seeds and 108.1 mg/kg DW in *Majhoul* seeds. Among the microelement iron was found at high level with concentration changed between 70.3 mg/kg DW in *Bousthammi* seeds and 27.7 mg/kg DW in *Boufgous* seeds followed by zinc (8.8–14.7 mg/kg DW), manganese (5.5–11.0 mg/kg DW) and copper (4.8–8.3 mg/kg DW) was the lowest microelement. This variation within minerals content of analyzed date seeds varieties may be due to variety, climatic conditions, soil type, water for irrigation, and fertilizer. Our results are in agreement with those reported by Al Juhaimi et al. (2012), Rahman et al. (2007) and Habib and Ibrahim (2009) with a slight difference. As shown in Table 5 below, the consumption of 100 g of date seeds can provide over 14% of the recommended daily allowance of magnesium, iron, copper, and manganese and more than 7% of zinc, however it covers less than 4% of calcium recommended daily allowance referred by Trumbo et al. (2002). Micronutrient deficiencies are a major public health problem in many developing countries, with infants and pregnant women especially at risk because they need adequate micronutrients to maintain normal growth and development (Batra and Seth, 2002; Rush, 2000). This important content on microelement can encourage food industries to use the flour of date seed to fortify their products.

Table 6 Phenolic and flavonoid content and antioxidant activity of date seed.

Varieties seeds	Antioxidant activity			Total phenolic content (g GAE/100 g DW)	Flavonoids content (RE/100 g DW)
	FRAP (mmol TE/100 g DW)	DPPH (g/L)	ABTS (mmol TE/100 g DW)		
Boufgous seed	14.299 ± 0.275	0.166 ± 0.004	4.807 ± 0.055	2.697 ± 0.036	1.224 ± 0.03
Bousthammi seed	22.863 ± 0.358	0.112 ± 0.005	8.021 ± 0.077	5.342 ± 0.071	1.844 ± 0.018
Majhoul seed	10.966 ± 0.339	0.133 ± 0.004	5.287 ± 0.129	3.078 ± 0.041	1.659 ± 0.022

Values in average ($n = 5$) ± SE. Averages, in the same column, with different letters are significantly different using post hoc PLSD of Fisher tests ($p < 0.001$).

Table 7 Correlation phenolic and flavonoid content with antioxidant activities.

	Phenolic	Flavonoids	FRAP	DPPH	ABTS
Phenolic	1.000				
Flavonoids	0.813	1.000			
FRAP	0.915	0.516	1.000		
DPPH	−0.856	−0.982	−0.599	1.000	
ABTS	0.998	0.816	0.913	−0.860	1.000

3.6. Phenolic and flavonoids content

The level of phenolic and flavonoids content of date seeds is significantly different ($p \leqslant 0.05$) as presented in Table 6. The highest contents on phenolic (5342 mg GAE/100 g DW) and flavonoid (1844 mg RE/100 g DW) were observed in *Bousthammi seed,* whereas the lowest amount of both phenolic (2697 mg GAE/100 g DW) and flavonoids (1224 mg RE/100 g DW) was found in *Boufgous seed*. Our data of analysis confirm previous results reported by Mistrello et al. (2014) who found that total phenol and flavonoids contents range between 2058–2983 mg GAE/100 g FW and 1271–1932 mg CE/100 g FW. However the present results are much higher than those reported by Al juhaimi et al. (2012) who found phenolic content ranging between 1.98 and 4.65 mg GAE/100 g of seed. This variation within may be owing to variety, growing condition, maturity, season, geographic origin, fertilizer, diseases, soil type and storage conditions as well as extraction system as shown in the results of Ardekani et al. (2010).

3.7. Antioxidant activities

The antioxidant activity was estimated using three in vitro tests: the FRAP assay based on the reduction of a ferric-tripyridyl triazine complex to its colored ferrous form in the presence of antioxidants, ABTS and DPPH assay based on the ability of antioxidant to scavenge 2,2′-azino-bis (ethylben zothiazoline-6-sulfonic acid) (ABTS+) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical cations. Table 6 summarizes the results of antioxidant power, which showed a significant difference ($p \leqslant 0.05$) between date seeds varieties. *Bousthammi* seeds exhibited the highest level of antioxidant activity based on FRAP assay (22.86 mmol TE/100 g DW), ABTS assay (8.021 mmol TE/100 g DW) and DPPH assay (IC50 = 0.112 g/l), the concentration of antioxidant which reduces the free radical DPPH$^{\cdot}$ about 50%. *Boufgous* seeds showed the lowest level of antioxidant activity based on DPPH and ABTS and the lowest level of antioxidant activity based on FRAP method was shown in *Majhoul* seed.

The analysis of correlation ($p \leqslant 0.05$) between antioxidant activity and phenolic content as well as flavonoid content indicted a strong correlation $IC50^{DPPH}$/phenolic content ($r = -0.856$), FRAP/phenolic content ($r = 0.915$), ABTS/phenolic content ($r = 0.998$), $IC50^{DPPH}$/flavonoid content ($r = -0.982$) and ABTS/flavonoid content ($r = 0.816$). However, moderate correlation observed between FRAP/flavonoid content ($r = 0.516$) is shown in Table 7.

The highest correlation between assays was observed between ABTS and FRAP ($r = 0.91$) and the lowest correlation was between FRAP/$IC50^{DPPH}$ and ($r = -0.599$).

The different antioxidant levels obtained from the assays may reflect a relative difference in the ability of antioxidant compounds in the extracts, to quench aqueous peroxyl radicals and to reduce $ABTS^{+}$, the DPPH free radical and ferric iron in in vitro systems (Thaipong et al., 2006).

4. Conclusion

The high amounts of minerals, antioxidants, fatty acids profile and the good proximate composition require a high valorization of this by-product using it as ingredient to enhance the nutritional value of some functional foods for human and animals' consumption as well as using its oil on pharmaceuticals, cosmetics and other formulations.

Conflict of interest statement

The authors declare no conflict of interest relating to the material presented in this article.

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