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Chemical Composition and Antioxidant Activity of Seed oil of Two Algerian Date Palm Cultivars (*Phoenix dactylifera*)

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The fatty acid composition of date seed oil from two different date palm (*Phoenix dactylifera* L.) cultivars, locally known as Degla-Baïdha and Tafezouine, were investigated. GC analysis revealed the presence of five dominant fatty acids: oleic C18:1 (46.51; 39.15%), lauric C12:0 (22.1; 28.5%), myristic C14:0 (10.7; 11.4%), palmitic C16:0 (9.6; 8.7%) and linoleic C18:2 (6.9; 6.1%). The oils was characterised by a low content of tocopherols (0.53; 1.41 µg/g). The antioxidant activity of the oils was investigated using the DPPH•(1,1-di-phenyl-2-picryl-hydrazyl) scavenging assay. The oils had a weak bleaching effect on DPPH• free radicals. This study showed that the qualities of the tested oils are highly comparable with those of some commercial seed oils of other plants. Furthermore, a statistical analysis using the hierarchy ascendant classification method was conducted in order to highlight the similarities and/or the differences regarding the contents of the main fatty acids found in some common plants and in the five most famous cultivars of *Phoenix dactylifera* of south eastern Algeria (Tafezouine, Degla-Baïdha, Deglet-Nour, Ghars, Tamdjouhert).

Keywords: Date seeds, Oil, Fatty acids, Phenolic content, Tocopherols, DPPH assay, Hierarchy ascendant classification (HAC).

Date pits of *Phoenix dactylifera* L. are a waste product of many date fruit processing plants. Studies aiming to extract and characterize some of the different fractions of date seeds have been limited to the oil, polyphenols and dietary fiber [1, 2]. Therefore, the aim of the present work was to determine the fatty acid and tocopherol compositions of the date seeds of two different cultivars (Degla-Baïdha (DB) and Tafezouine (Taf)) growing in the same region and that had not yet been investigated. This study also involved the evaluation of the total phenolic compounds and the antioxidant activities of the oil extracts. In addition, the physicochemical characteristics of these and other oils were also determined (Deglet-Nour, Ghars and Tamdjouhert). This study is an extension of some previous work on *Phoenix dactylifera* [3, 4].

At 20°C, all the seed oils examined were semi solids. The color of the oils varied from green-yellow to brown-yellow. The physicochemical properties of the oils extracted from date seeds and the amounts (yields) of oils are listed in Table 1. The crude fat (total lipids) content of the seeds was 5.6% for the DB cultivar and 5.4%, w/w, for the Taf cultivar. These results are similar to those found in our earlier study [3] in which the contents varied between 5.0 and 6.08% for the other cultivars. A comparison with some previous reports shows that the oil contents of our cultivars were very close to those of 'Khalti' and 'Kentichi' from Tunisia [5, 6], at the same time, they were lower than those of some other varieties [2, 7]. This evaluation indicated that the date seeds cannot be considered as an oleaginous seed, such as peanut, olive and sunflower seeds, which possess 30-40% oil. Although date seeds cannot be considered as oil-bearing seeds, their oil contents were in the range of other vegetable materials that are used as health components or have industrial or pharmaceutical applications, as in the case of wheat germ, acorn fruits and sorghum seeds, which have "less than 10% fat content" [8, 9]. Furthermore, all the determined physiochemical proprieties were similar to those found in our earlier study [3].

Table 1: Physicochemical characteristics of seed oils of *Phoenix dactylifera*.

Parameter	Cultivars	
	DB	Taf
Iodine value (g Iodine/100 g)	72.9±1.3	74.4±0.5
Saponification value (mg KOH/g)	209.8±2.4	207.8±2.6
Acid value (mg KOH/g)	1.4±0.3	1.3±0.2
Refractive index n_d^{20} (20°C)	1.4800	1.4801
Specific gravity d^{20} (20°C)	0.905	0.921
Total oil content % (g/100 g)	5.6±0.8	5.4±0.6

DB: Degla-Baïdha, Taf: Tafezouine.

The refractive index and relative density values for the date seed oils were comparable with those of other vegetable oils; so, these results suggest that the oils contain an important amount of unsaturated fatty acids. The acid value was low in the two oils, which indicates that the oils contain a small amount of free fatty acids; this low value could be due to the small period of exposure of the seeds to air during the maturity of the fruits. The low free fatty acid (FFA) content of the oils shows that they are edible and could have a long shelf life. Iodine values of the oils were 72.9 for DB and 74.4 (g I_2 /100 g) for Taf. The relatively high iodine value in both oils could be a sign of the presence of many unsaturated bonds (unsaturated fatty acids) and can thus be grouped as drying oils. The saponification values were very similar for the two studied varieties being 209.8 and 207.8 mg KOH/g for DB and Taf, respectively. Since there is a reverse relationship between saponification value and the weight of fatty acids in oils, it can be assumed that the oils hold fatty acids with 16-18 carbon atoms. Finally, the values of the physicochemical properties of these oils are in the range of those reported for the majority of vegetable oils.

The FAME(s) compositions of the two oil varieties of date seeds are listed in Table 2. Ten fatty acids were identified in the seed oils. Individual percentages of each fatty acid are given in Table 2. The identified saturated fatty acids were capric, lauric, myristic, palmitic, stearic and arachidic acids. Lauric acid was the major saturated fatty acid constituent, with percentages of 22.1 and 28.5%

for DB and Taf, respectively, followed by myristic, palmitic and stearic acids. Arachidic acid was not detected in the seed oil of the DB variety. The unsaturated fatty acids, oleic and linoleic acids, were detected in both oils. Oleic acid was the dominant fatty acid in the oils, with percentages of 46.5 and 39.2% in DB and Taf varieties, respectively. For linoleic acid, the contents were 6.9 and 6.1% for the DB and Taf varieties, respectively. Linolenic acid was detected only in the DB variety and in very small amount, that did not exceed 0.5 %. The most abundant fatty acids were oleic, lauric, myristic, palmitic and linoleic acids. Apart from some minor differences, the fatty acid profiles of the two date seed oils were much the same. The values of the U/S ratio (U/S = unsaturated fatty acids/saturated fatty acids) were 0.83 for Taf and 1.17 for DB. These values indicate that no class of fatty acids was dominant. These results are in general agreement with those reported [2, 3, 7, 10-13].

When we compared the unsaturation degree of the studied oils with the commonly consumed vegetable oils, the date seed oils had a relatively lower degree of unsaturation and a lower amount of linoleic acid. Despite this low level of unsaturation, date seed oils have potential use in human and/or animal diets.

Table 2: Fatty acid compositions of different cultivars of date seeds oils of *Phoenix dactylifera*.

Fatty acid	Cultivars	
	DB	Taf
Capric $C_{10:0}$	–	4.3±0.5
Lauric $C_{12:0}$	22.1±0.5	28.5±0.1
Myristic $C_{14:0}$	10.7±0.2	11.4±0.1
Palmitic $C_{16:0}$	9.6±0.3	8.7±0.3
Stearic $C_{18:0}$	3.4±0.4	1.9±0.2
Oleic $C_{18:1}$	46.5±0.4	39.2±0.1
Linoleic $C_{18:2}$	6.9±0.1	6.1±0.1
Linolenic $C_{18:3}$	0.3±0.1	–
Arachidic $C_{20:0}$	0.4±0.1	–
∑SFA	46.1	54.7
∑UFA	53.9	45.3
U/S	1.17	0.83

DB: Degla-Baïdha, Taf: Tafezouine. ΣSFA: sum of saturated fatty acids, ΣUFA: sum of unsaturated fatty acids. U/S: ratio "unsaturated/saturated" fatty acids.

The total phenolic contents, which comprise tocopherols, were determined for the five most famous varieties in the region of Ouargla (Degla-Baïdha, Tafezouine, Deglet-Nour (DN), Ghars (Gh) and Tamdjouhert (Tam)). The total phenolics, taking into account tocopherols, were determined by a modified Emmerie-Engel method [14]. The results were expressed as vitamin E equivalents (Table 3). The phenolic compounds may contribute directly to the antioxidant action. Our data indicate the possible presence of natural antioxidant phenolic compounds in all date seed oils (tocopherols and other phenolic compounds). The total phenolic contents of the oils, ranged from 0.64 to 1.27 mg/g (Table 3). The seed oil of the Gh variety had the highest content of phenolic compounds, while the Taf variety had the lowest (0.64 mg/g). In fact, the amount of phenolic compounds in the seed oil of the Gh variety was twice as much as that in the Taf variety.

Table 3: Total phenolic (with tocopherols) contents and antioxidant activity of different cultivars of seed oils of *Phoenix dactylifera*.

	DB	Taf	DN	Gh	Tam
Total phenolic content (mg/g)	0.85±0.001	0.64±0.001	0.91±0.002	1.27±0.002	0.72±0.001
IC_{50} (g/mL)	0.17±0.005	0.33±0.005	0.21±0.005	0.14±0.005	0.29±0.005

DN: Deglet-Nour, Gh: Ghars, DB: Degla-Baïdha, Tam: Tamdjouhert, Taf: Tafezouine.

Tocopherols constitute a family of lipophilic antioxidants that protect biological membranes against peroxyl radicals. Seed oils such as soybean, sunflower and peanut, which contain high concentrations of total tocopherols (four isomers α, β, γ and δ), are the major sources of vegetable oil in the world. The seed oils of the investigated dates revealed the presence of small amounts of tocopherols (Table 4). The Gh seed oil had the highest level of total tocopherols, at 2.76 µg/mg, followed by the DN variety (2.69 µg/mg) then Taf (1.43 µg/mg) and then the DB seed oil (0.53 µg/mg). For the Tam seed oil no tocopherols were detected. The major tocopherol in the seed oils of dates was the α isomer, which accounted for 62.5%, 62.2%, 47.2% and 42.8% of the total tocopherols in DN, Taf, DB and Gh varieties, respectively. On the other hand, the Gh and DB seed oils contained the highest relative concentrations of (β+γ)-tocopherols, which contributed 39.1 and 30.2% of the total tocopherols, respectively. The seed oils of date contained δ-tocopherol in percentages of 22.6, 17.4, 10.0 and 6.3% of the total tocopherols for the DB, Gh, DN and Taf, respectively.

The tocopherol concentrations in the date seed oils obtained in this study were much lower than those for oleaginous seeds. It is obvious that the total phenolic contents determined by spectrometric methods are much higher than those determined by HPLC (tocopherols only), *i.e.* the oils contain an important quantity of other phenolic compounds other than tocopherols which could interfere in the quantification of the total phenolic compounds using a spectrometric method. In fact, the Emmerie-Engel method is reported to detect all phenolic compounds present in the oil; also the ferric ions could be reduced by other constituents beside phenolic compounds, such as carotenoids and vitamin A present in the oils.

Total phenolic content and free radical-scavenging activity of seed oils showed an important linear correlation (R = 0.73). Furthermore, a very strong correlation has been found between the free radical scavenging activity and the percentage of total unsaturated acids (R = 0.966). These results can be explained by the fact that the tocopherols in vegetable oils are believed to protect polyunsaturated fatty acids from oxidation. According to the literature [15], α-tocopherol exhibits the highest biological vitamin E activity, whereas γ-tocopherol exhibits only ≈10–30% of the activity of α-tocopherol. Also, these well-known natural antioxidants are often correlated with a relatively high abundance of unsaturated fatty acids.

The DPPH assay was used to evaluate the ability of antioxidants to scavenge free radicals. The ability of oils to scavenge the $DPPH^{\bullet}$ radical was assessed on the basis of their IC_{50} value, defined as the concentration of oil that decreases the absorbance at 517 nm of the $DPPH^{\bullet}$ radical solution to half of its initial value. IC_{50} values of the ethanolic oil solutions are listed in Table 4. All the oils exhibited very weak radical-scavenging activities for the bleaching of the free radical of $DPPH^{\bullet}$. The IC_{50} values were in the range 0.14-0.33 g/mL. The lowest value was detected for the seed oil of the Gh variety, which corresponds to the highest antioxidant activity; while the highest value of IC_{50} was detected for the seed oil of the Taf variety (lowest antioxidant activity). The antioxidant activity of the oils deceased in the following order: Gh > DB > DN > Tam > Taf.

Table 4: Tocopherols composition of the seed oils of different cultivars of *Phoenix dactylifera*.

Tocopherols (µg/g)	DB	Taf	DN	Gh	Tam
α-tocopherol	0.25±0.01	0.89±0.03	1.68±0.15	1.18±0.12	nd
(β+γ)-tocopherol	0.16±0.02	0.44±0.01	0.73±0.07	1.08±0.05	nd
δ-tocopherol	0.12±0.01	0.09±0.01	0.27±0.01	0.48±0.02	nd
Total	0.53	1.43	2.69	2.76	–

DN: Deglet-Nour, Gh: Ghars, DB: Degla-Baïdha, Tam: Tamdjouhert, Taf: Tafezouine. nd: not detected.

In order to highlight the difference in the contents of the main fatty acids of *P. dactylifera* (different cultivars in Algeria) with other common plants (data provided from the literature), we have chosen the method of cluster analysis, using Ward's technique [16];

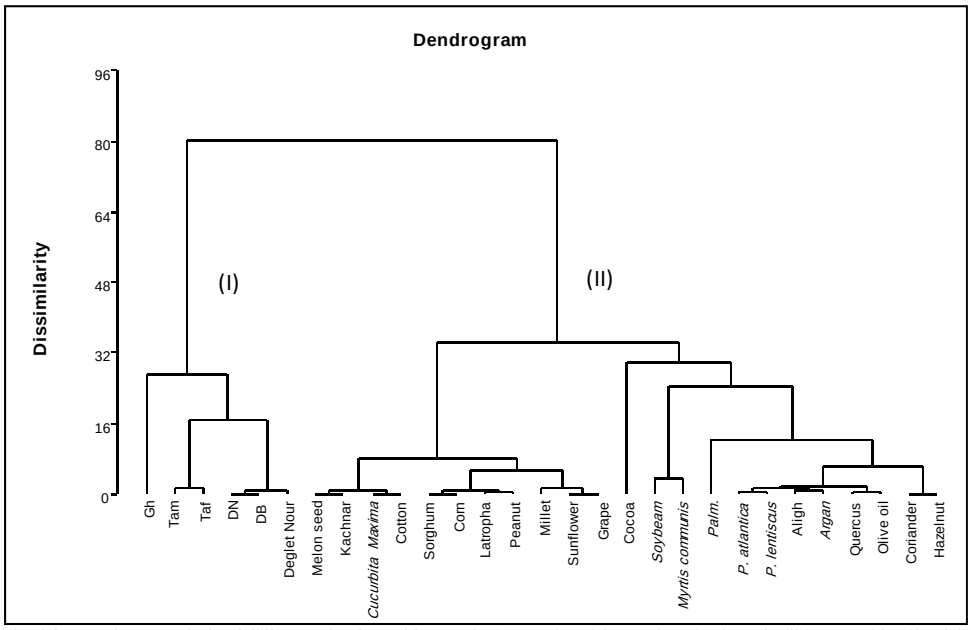

Figure 1: Dendrogram obtained from a cluster analysis of different Algerian cultivars of *Phoenix dactylifera* and a selection of edible vegetable oils of different plants. Samples are clustered using Ward's technique with a Euclidean distance measure.

13 fatty acids representing the main compounds in these plants (29 individuals) were employed. The results show the existence of two principal clusters (I and II) within the fatty acids of the individuals of the investigated plants (Fig. 1).Group (I) is referred to the Algerian and Tunisian *P. dactylifera* cultivars. This cluster shows that the seeds of Deglet-Nour from Algeria [3] are very similar to those from Tunisia [7] in terms of nutritional effects (fatty acids compositions values), and these seeds have similar main components as the seeds of DB [3]: ($C_{12:0}$: 17.8-25.6%; $C_{14:0}$: 9.84-10.7%; $C_{16:0}$: 9.1-10.9%; $C_{18:0}$: 3.1-5.67%; $C_{18:1}$: 41.3-42.5%; $C_{18:2}$: 6.9-12.2%). Moreover, Tam, and Taf cultivars are more alike. ($C_{10:0}$: 4.34-6.26%; $C_{12:0}$: 21.0-28.4%; $C_{14:0}$: 11.4-11.6%; $C_{16:0}$: 8.7-9.7%; $C_{18:0}$: 1.9-3.6%; $C_{18:1}$: 39.1-40.6%; $C_{18:2}$: 6.1-7.0%). However the Gh cultivar is different from the other cultivars in this group (I). This slight difference is due to the presence of $C_{14:1}$ in low quantity (1.14%), which was not detected for the other varieties in this first group. Moreover, it can be seen clearly that the date palm cultivars are clearly separated from the other plants. The second group (II) can be separated into two subgroups SG-1 and SG-2. The first (SG-1) is referred to the following plants: melon-seed, kachnar, *Cucurbita maxima*, cotton, sorghum, corn, latropha, peanut, millet, sunflower and grape. The rest of the plants belong to the second subgroup. The main differentiation of these two groups is attributed mainly to the very low content of both $C_{12:0}$ and $C_{14:0}$ in the second group with percentages ranging from 0.0-5.81% and 0.0-3.12%, respectively, While the content of $C_{12:0}$ and $C_{14:0}$ in the first group are 17.7-28.4% and 9.81-11.6%, respectively. It can be seen from Figure 1 that melon-seed, kachnar, *Cucurbita maxima* and cotton have similar contents. The same observation is extendable to sorghum, corn, latropha and peanut. Millet, sunflower and grape are also similar. SG-1 is characterised by the following ranges of contents: $C_{16:0}$: 5.5-25.7%; $C_{18:0}$: 1.27-13.6%; $C_{18:1}$: 12.72-44.7%; $C_{18:2}$: 32.8-68.7%). For SG-2, *P. atlantica*, *P. lentiscus*, aligh, argan, quercus and olive oil are very similar. Coriander and hazelnut are also much alike. SG-2 is characterised by the following ranges of contents: $C_{16:0}$: 0.0-43.7%; $C_{18:0}$: 0.0-44.6%; $C_{18:1}$: 27.5-82.12%; $C_{18:2}$: 4.9-47.0%; $C_{18:3}$: 0.0-8.7%).

Finally, the results found in this investigation should be completed by additional studies aimed at the assessment of the biological activities and identification of the individual phenolic compounds of these oils.

Experimental

Seed material: Two different Algerian date palm fruit cultivars: Degla-Baïdha (DB) and Tafezouine (Taf) were obtained from a palm grove in the Ouargla region (south-eastern Algeria). The different varieties were identified within the Agronomic National Institute of Ouargla. The seeds (pits) were manually separated from the flesh. Their relative percentage weight compared with the weight of the fresh fruits was 20.5% for the Degla-Baidha variety, and 15.0% for the Tafezouine variety. Then, the seeds were rinsed clear of any flesh by water and dried for 48 h at 60°C, as described [10], and then ground to a fine powder to pass through a 1-2 mm mesh, as described [2, 7].

Extraction of the date-seed oils: The dry seed powder was extracted in a Soxhlet apparatus with *n*-hexane for 6 h. The solvent was removed using a rotary vacuum evaporator at a temperature not exceeding 40°C and the oils obtained were stored in a freezer at +4°C until analysis.

***Determination of physical and chemical indices of the oils*:** Acid value (AV), saponification value (SV), iodine value (IV), relative density (d^{20}) and refractive index (n_d^{20}) were determined according to the procedures described by AFNOR [17].

Determination of fatty acid composition: Fatty acid methyl esters (FAMES) of the oils were prepared using the method described by AFNOR [17] and then analyzed using a Delsi GC apparatus equipped with a flame ionization detector (FID) and a capillary column (MEGA-10, 25 m×0.32 mm×0.25 µm). The column temperature was programmed from 150 to 200°C at 2°C/min and the injector and detector temperature were set at 250°C. Helium was the carrier gas. The identification of the peaks was achieved by retention times and by comparison with authentic standards analysed under the same conditions.

Determination of the total phenolic contents (together with tocopherols) in the oils: The total phenolic (including tocopherols

and some other phenolic compounds) contents of the oils was determined by the spectrometric method described in reference [14] with a few modifications. This method is based on the reduction of Fe^{3+} to Fe^{2+} by phenolic compounds. From the standard solution of α-tocopherol in dichloromethane we prepared aliquot solutions with different concentrations (0.005, 0.010, 0.015, 0.020, 0.025, 0.030, 0.035, 0.040 and 0.045 g/L. To 2 mL of each solution was added 1 mL of 1,10-phenantroline reagent (0.4% in ethanol), then 1 mL of ferric chloride reagent (0.12% in ethanol) was added and the mixture was shaken for 5 min. The absorbance of the mixture was read at 510 nm. A blank was run, using 2 mL of dichloromethane, 1 mL of 1,10-phenantroline reagent and 1 mL of ferric chloride reagent. The above described procedure was followed by using sample solutions of oils. The total tocopherol in the oils was calculated from the regression equation of the standard curve.

Tocopherol analysis: The tocopherol compounds were quantified using a Waters Alliance 2690 HPLC system, equipped with a quaternary pump, a thermostatted column compartment and a fluorescence detector. A Licsopher RP-18 column (250×4.6 mm, 5 µm thicknesses, Merck) was used with methanol: acetonitrile (70:30 v/v) as mobile phase, with an isocratic elution flow of 1 mL/min. Quantification was carried out from a calibration based on standard tocopherols. β-Tocopherol was not resolved from γ-tocopherol by RP chromatography. For this reason, we described β and γ-tocopherols together.

DPPH radical–scavenging assay: The DPPH radical scavenging assay was conducted according to the method of [18]. Briefly, 1 mL of ethanolic DPPH solution was mixed with 1 mL of the samples dissolved in ethanol at different concentrations. The reaction mixture was shaken and incubated in the dark at room temperature for 30 min, and the absorbance was read at 517 nm against a blank. Control "α-tocopherol" was prepared in a similar way as for the test group except for the replacement of the antioxidant solution with the corresponding extraction solvent. For this control, the inhibition percentage was found to be: IC_{50} = 0.034 g/L. The inhibition of the DPPH radical by the sample was calculated according to the following formula: Inhibition % = $(1 – A/A_0).100$ %
Where A_0 and A are the absorbance values of control and of the tested sample, respectively.

Cluster analysis: Cluster analysis was performed using hierarchical clustering (Ward's technique) with Euclidean distance measure[16]. The calculus was performed using a set of data composed of 24 different plants and 13 variables (main compounds reported > 2.5% are: $C_{10:0}$, $C_{12:0}$, $C_{14:0}$, $C_{14:1}$, $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$, $C_{20:1}$, $C_{22:0}$).

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