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DATE PALM *PHOENIX DACTILIFERA L.* SEED OIL: VARIETY EFFECTS ON PHYSICOCHEMICAL CHARACTERISTICS, FATTY ACID COMPOSITION, STEROL AND TOCOL CONTENTS

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ABSTRACT

The date palm (*Phoenix dactylifera* L.) grows in the world's arid and semi-arid regions, particularly in the majority of Middle Eastern countries. It has played an important role in the survival of many ancient civilizations. It is regarded as a valuable nutrient, but it also plays an important role in the economies of date-producing countries. Date palm seeds are commonly described as waste after consuming their pulp by industries or individuals. However, date pits contain a valuable source of edible oil characterized by a high content of unsaturated fatty acids, sterols, tocopherols, and tocotrienols. The main purpose of this study was to analyze the chemical composition and quality parameters of Date Seed Oil (DSO) extracted from ten native date palm seed (*Phoenix Dactilifera L.*) varieties and compare them to the literature results. The choice of the seed variety was based on popularity and quality. The lipid extraction was carried out by a soxhlet apparatus using a standard solvent with a seed isolated from a matured date. The yield ranged from 2.94% to 8.06% depending on the variety. Gas chromatography coupled to mass spectroscopy (GC/MS) and high-performance liquid chromatography (HPLC) were used to determine the composition of the fatty acids, sterols, and tocols (tocopherols and tocotrienols) in the extracted oil. Our results indicate that DSO is rich in poly and unsaturated fatty acids (PUFA+MUFA = 48.89% to 60.77%) with oleic acid (C18:1) as the predominant fatty acid (from 52.60% to 42.13%). On other hand, eight sterols were identified and the mean content ranged from 346.97 to 816.60 mg/100g of oil. Also, a high amount has been recorded in tocols ranging from 1937.14 µg/g to 3844.06 µg/g. Indeed, the predominant tocols in DSO were alpha-tocotrienols and alpha-tocopherols. Likewise, the quality parameters indicate that DSO has excellent thermal and oxidative stability and is generally safe to consume. In fact, the peroxide value (PV) recorded a mean of 3.96 to 6.33 meq O_2/kg. The iodine value of DSO samples goes from 48.84 to 60.59 g I_2/100 g oil. The saponification value (SV) changed between 214.85 and 225.59 mg KOH/g of oil. However, the coefficients of extinction K232 and K270 were lower than the specified limitations. According to the findings, date seed has the potential to be used as a renewable resource while also adding value to pharmaceutical, cosmetic, food, and agricultural products.

Keywords: Chemical composition; Date seed oil; Oil pigments; Quality parameters; Tocopherols and tocotrienols

INTRODUCTION

Date palm tree (*Phoenix dactylifera L.*) has usually performed a crucial financial and social function in arid and semiarid areas. The date fruit and kernel possess a considerable number of proteins, sugars, dietary fibers and minerals. Although the kernel can be considered as a potential source of edible oil (2.0-13.2%) (**Abdalla et al., 2012**) compared to the flesh (0.2-0.5%) (**Al-Shahib & Marshall, 2003**). Even though, it is generally used as animal feed complementary, in the propagation of date palms, as common soil fertilizer due to the high content of minerals, or as a substitute for decaffeinated coffee, the Arabs commercialized it in two types of product, plain or mixed with coffee (**Ali-Mohamed & Khamis, 2004**).
According to FAO's data, the total world date production has exceeded 9 million tons, and Morocco has produced more than 101.537 tons (**FAO, 2019**). Over the past 30 years, the world date production has increased significantly, from 3.430.585 tons in 1989 to 5.948.312 tons in 1999, then from 7.184.082 tons in 2009 to 9075446 tons in 2019. Unfortunately this international development in date production is in parallel accompanied with more than 30% of the lost of dates owing to the harvesting, storage or conditioning process (**Borchani et al., 2010**).
Since date seeds are also recognized to possess treasured bioactive compounds, it is highly desirable to use this by-product. Indeed, it has many medicinal and therapeutical effects. A study of Graham et al.(**Graham et al., 2000**) used DSO as a liniment for the treatment of indolent tumors. Also, DSO shows antimicrobial effects against alpha and beta hemolytic Streptococci, *Escherichia coli*, *Staphylococcus aureus* and *Aspergillus fumigatus* (**Barmak et al., 2011**).
The review of the literature found that no previous research on Moroccan date palm seed oil had been conducted to evaluate its chemical composition in sterol and tocol, physicochemical parameters, or any other potential uses. The primary goal of this study was to examine the tocol (tocopherol and tocotrienol) and phytosterol compositions, fatty acids, and physicochemical profiling of Phoenix dactilifera L. seed oils from ten native date varieties. The second purpose was to make a comparison between the results of date seed varieties and the data from the literature. Then utilize a correlation matrix, the Principal Components Analysis (PCA) and Hierarchy Cluster Analysis (HCA) to better understand the similarities and differences between the final data.

MATERIAL & METHODS

Preparation of plant extracts

Ten varieties of date seed were used for our study. All the collected dates were in different and distinctive regions in Sous-Massa, Daraa tafilalet, and Oriental in Morocco at the Tamr stage. The seeds were isolated from the fruits, soaked in distilled water, and well washed to shake off any leftover date fruit. Then they were dried at 50°C for a night, crushed in a swing type electric grains herbal powder miller and preserved in a freezer (-16 °C) until extraction. The extraction was carried out using the soxhlet method; about 60 g of powdered seed were used for each extraction with an apolar solvent to extract vegetal oil. After the extraction, the vegetal oil was also stored in a freezer until analysis.

Phytochemical Determination

Determination of oil pigments: chlorophyll and carotenoid content

Approximately 7.5 grams of DSO were dissolved in 25 mL of cyclohexane. The solution was vortexed and then the absorbance was measured in a UV spectrophotometer (LLG-uniSPEC 2) for the chlorophyll content at 670 nm and

for the carotenoid content at 470 nm using a quartz bowl with a 1 cm path length. The obtained values were calculated from the subsequent equations (1) and (2) and were expressed in mg/kg (**Suri et al., 2020**).

$$Chlorophylls\ (mg\ kg^{-1}) = \frac{Abs_{670} \times 10^6}{613 \times 100 \times curvelenght} \quad (1)$$

$$Carotenoids\ (mg\ kg^{-1}) = \frac{Abs_{470} \times 10^6}{2000 \times 100 \times curvelenght} \quad (2)$$

Physicochemical analysis of DSO

To control the quality of the extracted oil, a recommended practices by the American Oil Chemists Society (AOCS) has been conducted. The most frequently used parameters to measure the physicochemical properties of vegetal oils are content of peroxide value, free fatty acid (FFA), saponification value (**Mofijur et al., 2017**), the specific UV extinction coefficients (K232 and K270) and the iodine value (AOCS, Official Methods and Practices of the AOCS, AOCS Press, Champaign, USA, Fifth edition, 1998., n.d.).

DSO's chemical composition of Fatty Acids (FA), sterols and tocols

DSO's composition of FA

The fatty acid analysis was proceeded through the determination of fatty acid methyl esters by gas chromatography using the EEC/ 2568/91 method. The chromatograph was coupled by a CPWax 52CB column (30 m × 0.25 mm) with an initial and final of 170 °C and 230 °C, and gradually increased with 4 °C/min. The rating flow was 1 mL/min using a carrier gas (helium). The Varian Star Workstation v 6.30 (Varian Inc., Walnut Creek, CA, USA) was used to process the results, which were expressed as a percentage of all fatty acids detected in DSO (**Ibourki et al., 2021**).

DSO's composition of sterols

According to ISO 6799, the phytosterols of DSO were determined by a standard method. The chromatograph (VARIAN CP-3800) uses capillary gas chromatography with an apolar column (30 m 0.32 mm, DI: 0.25 m, phase: CPSIL8CB) and a divider injector type 1079 (T: 300 °C), a FID (T: 300 °C), and helium gas as a carrier with a flow rate of 1.5 mL/min (**Gharby et al., 2014**).

DSO's composition of tocols

The determination of vitamin E's isomere has been performed by the standard method ISO 9936 using High-performance liquid chromatography (HPLC) equipped with a silica column (25 cm × 4 mm) and a fluorometric detector. The wavelengths of excitation and emission were from 290nm and 330nm respectively. The eluent was prepared from a mixture of isooctane and isopropanol (99:1) (V/V), with a flow rate of 1.2 mL/min. The quantification was carried out using an external standard and referring to the American Oil Chemists' Society (AOCS) data reference (**Nasri et al., 2021)**.

Statistical analysis

Data Analysis

XLSTAT 2014 software was used for the Pearson correlation, PCA and HCA (**Zielinski et al., 2014**). To analyze the variance and the statistical significance of the results, a Tukey test at a confidence level of 95.00% has been conducted. The data was expressed as mean standard error and analyzed using IBM SPSS Statistics 21 software.

Analyze the Principal Components (PCA). Cluster Analysis in Hierarchy (HCA)

The purpose of principle component analysis in this study is to establish the existence of a correlation between total sterol, total tocopherol, Chlorophyll, Carotenoid, Linoleic (C18:2), and linolenic (C18:2) acids in various Palm dates seeds varieties (10 samples), then between total sterol, total tocopherol, chlorophyll, carotenoid, linoleic (C18:2), and linolenic (C18:3). The results of Physicochemical parameters quality (Acid Value, Saponification Value, K270, K232 and Iodine Value, Peroxide Value) and total sterol, total tocopherol, chlorophyll, carotenoid, Linoleic (C18:2) and linolenic (C18:3) acid, which represented 12 variables, as well as those of the 10 samples oils from Palm dates, were subjected to principal component analysis. HCA was used to pursue the interrelatedness between all samples oils as a cluster characteristic, moreover, the dendrogram was shaped using the Ward cluster technique, and the squared Euclidean distance was considered as a coefficient of similarity.

Correlation Matrix

The PCA was carried out on a matrix that contained all of the data from the various Physicochemical characteristics of the quality, including total sterol, total tocopherol, Chlorophyll, Carotenoid, Linoleic (C18:2) and linolenic (C18:3) acids. The ten samples of Palm dates oils reflect the individuals.

RESULTS AND DISCUSSION

Physicochemical analysis of DSO

DSO's quality parameters

The analysis of DSO's yield and quality parameters has been regrouped in Table 1. The extraction yield of DSO by soxhlet method using an apolar solvent was ranged from 2.94% to 8.06%. Likewise, Ben-Youssef et al. (**Ben-Youssef et al., 2017**) and Jadhav and al. (Jadhav et al., 2016) used the same experimental conditions, there extraction yield was 4.44% and 8.5% respectively.

Table 1 Physicochemical parameters of DSO

QI	*DN1*	*DN2*	*DN3*	*DN4*	*DN5*	*DN6*	*DN7*	*DN8*	*DN9*	*DN10*
DSO's Yield (w/w) (%)	3.46±0.64^{a}	3.59±0.48^{a}	4.39±0.64^{b}	3.62±0.53^{a}	4.25±0.67^{b}	4.97±0.46^{c}	2.94±0.40^{a}	4.28±0.49^{b}	4.12±0.15^{b}	8.06±0.95^{c}
Carotenoids (mg/kg)	5.14±0.15	6.60±0.03	6.85±0.04	7.88±0.03	4.88±0.07	7.95±0.08	6.20±0.65	6.83±0.11	2.21±0.04	2.34±0.04
Chlorophylls (mg/kg)	10.67±0.15	15.30±0.03	15.74±0.03	17.77±0.07	10.04±0.08	20.81±0.16	13.49±0.09	14.42±0.0.26	5.23±0.11	3.44±0.06
FFA %	2.54±0.06^{a}	2.27±0.02^{b}	2.11±0.03^{c}	2.45±0.03^{a}	2.20±0.08^{b}	2.37±0.02^{d}	2.38±0.02^{d}	1.52±0.11^{e}	2.48±0.03^{a}	1.10±0.06^{f}
Peroxide value (meq O_2/kg)	5.43±0.28^{a}	6.33±0.33^{b}	5.80±0.60^{a}	5.54±0.18^{a}	5.23±0.23^{a}	4.48±0.15^{c}	3.96±0.12^{d}	3.88±0.23^{d}	6.23±0.24^{b}	5.14±0.42^{a}
Saponification value (mg KOH/g oil)	222.08±1.5^{a}	222.81±1.82^{a}	220.99±2.68^{a}	218.54±1.88^{b}	216.21±2.13^{b}	225.59±2.34^{c}	225.45±1.99^{c}	223.79±3.68^{c}	221.56±2.37^{a}	214.85±2.88^{b}
Iodine value (g I_2/100 g oil)	56.66±3.47^{a}	54.12±3.34^{a}	57.87±3.65^{ab}	59.76±3.76^{b}	60.59±3.86^{b}	48.84±3.12^{c}	50.30±3.18^{c}	50.87±3.25^{c}	55.89±3.52^{a}	57.68±3.53^{ab}
K_{232}	1.72±0.05^{a}	2.04±0.06^{b}	1.28±0.02^{c}	2.43±0.03^{d}	2.13±0.03^{e}	2.42±0.04^{d}	1.58±0.03^{f}	1.62±0.04^{f}	1.65±0.03^{f}	1.82±0.04^{g}
K_{270}	1.26±0.06^{a}	1.33±0.03^{a}	0.78±0.02^{b}	2.36±0.02^{c}	1.04±0.03^{d}	2.03±0.04^{e}	1.41±0.03^{f}	1.28±0.02^{a}	2.14±0.03^{g}	1.59±0.02^{h}

The results of chlorophyll and carotenoid content are expressed in Table 1. The highest amount of chlorophyll was recorded for bousthammi and Iklan varieties with 20.81 mg/kg and 17.77 mg/kg, then Boufkouss, Lkhat, Jihel, and Ablouh recorded a similar value of 15.74 mg/kg, 15.30 mg/kg, 14.42 mg/kg and 13.49 mg/kg, followed by Mejhoul and Bouslikhen with 10.67 mg/kg and 10.04 mg/kg, whereas the poorest amount was registered for both varieties of Aziza and Lkenz with 5.23 mg/kg and 3.44 mg/kg.

Likewise, the carotenoid value shows approximately the same classification of varieties as chlorophyll's result. Indeed, Bousthammi and Iklan recorded a high and similar amount with 7.95 mg/kg and 7.88 mg/kg, then the mean value was 6.85 mg/kg, 6.83 mg/kg, 6.60 mg/kg, 6.20 mg/kg respectively for Boufkouss, Jihel, Lkhat, Ablouh. Then Lkenz and Aziza with 2.34 mg/kg and 2.21 mg/kg, were the last varieties in the classification.

The FFA or acid value has an efficient impact on the oil's quality, especially the organoleptic aspect. Its analysis of DSO recorded a similar value for all varieties from 2.11% to 2.54% except for the variety of Jihel (DN8) and lkenz (DN10) with 1.52% and 1.10% respectively. These FFA's results of DSO were similar to those reported by Nehdi et al.(**I. A. Nehdi et al., 2018**) and Devshony et al.(**Devshony et al., 1992**). Therefore, the peroxide value reported by Nehdi et al.(**I. A. Nehdi et al., 2018**) was higher than our results, with 16 meq O_2/100 g and 25 meq O_2/100 g for Deglet Nour and Allig DSO respectively, while our registered values were from 3.96 meq O_2/100 g to 6.33 meq O_2/kg. However, the low value of peroxide (less than 30 meq O_2/100 g) indicates that DSO is fresh and has a low probability of autoxidation, which implies that it can be considered as safe for being consumed by humans (**Gotoh & Wada, 2006**). Although a study of Abdalla et al.(**Abdalla et al., 2012**), Nehdi et al.(**I. A. Nehdi et al., 2018**) and Al Juhaimi et al.(**Al Juhaimi et al., 2018**), they recorded a similar and lower value of peroxide ranging from 4.8-7.4 meq O_2/kg, 3.26-5.62 meq O_2/kg and 1.14-3.44 meq O_2/kg respectively. These variations can occur due to various factors like the storage or conditioning process, exposure to light, the degree of the unsaturated fatty acids present in oil, and the content of metals or bioactive compounds that protect against or may catalyze oxidation processes (**Choe & Min, 2006**).

Saponification value (SV) pertains to the mass of potassium hydroxide in mg that neutralizes all the free fatty acids and saponifies the esters present in a gram of oil (**Mofijur et al., 2017**). According to Bart et al.(**Bart et al., 2010**) SV of coconut oil was 242-263 mg KOH/g oil, for babassu it was 241-253 mg KOH/g oil, palm kernel it was 240-257 mg KOH/g oil, and for palm it was 200-205 mg KOH/g oil. Our recorded low and high value was 214.85 mg KOH/g oil for the variety Lkenz (DN10) and 225.59 mg KOH/g oil for the variety Bousthammi (DN6). A high SV can be translated as a high number of carbon atoms of the fatty acids present in DSO, which means after hydrogenation, DSO can be a substitute for conventional oils (**I. Nehdi et al., 2010**).

The iodine value is a measurement of the unsaturation degree of fats and oils; it's expressed as the number of grams of iodine per 100 g of sample. Our results Iodine value of DSO was ranged between 48.84-60.59 g I_2/100 g oil, for the variety of Bousthammi (DN6) and Bouslikhen (DN5) respectively, these values indicate that DSO is classified as a highly unsaturated and a non-drying oil (**Mohammed et al., 2003**). In the literature (**Lidefelt, 2007**), the iodine index from other conventional oils was 34-40 g I_2/100 g oil for cocoa butter, 50-55 g I_2/100 g oil for Palm oil, 100-115 g I_2/100 g oil for cotton seed, 118-141 g I_2/100 g oil for sunflower seed, and 48-60 g I_2/100 g oil for DSO.

To study the presence of oxidation compounds, a measure of the K232 and K270 known as the specific UV extinction coefficients has been conducted. They are also suitable for determining the quality and conservation process of oil (**Rodrigues et al., 2015**). The K232 is related with the presence of initial products of oxidation (conjugated hydroperoxides), the mean value was from 1.28 to 2.43. The K270 is related to the presence of final oxidation products (FFA, aldehyds and ketones), K270 value was from 0.78 to 2.14, which is lower than the specified limitations (≤ 2.50). Therefore, in our study, DSO of all varieties didn't record a high content of oxidation products.

DSO's chemical composition of Fatty Acids (FA), sterols and tocols

DSO's composition of FA

The chemical composition of the fatty acids of the 10 studied date seed varieties is shown in Table 2. Overall, 14 fatty acids were detected in all of the 10 studied varieties of date seed. Eight of them were saturated fatty acids, called, caprylic ($C_{8:0}$), capric (C10:0), lauric (C12:0), myristic (C14:0), palmitic (C16:0), margaric (C17:0), stearic (C18:0), and arachidic (C20:0) acids. Then the four monounsaturates were palmitoleic (C16:1), margaroleic (C17:1), oleic (C18:1), and gadoleic (C20:1) acids. While the two left were polyunsaturated fatty acids, named, Linoleic (C18:2) and linolenic (C18:3) acids. The results of fatty acid analysis of all Date Seeds Oil DSO varieties show a slight variation in Table 3.

Table 2 Fatty acid composition (%) of DSO.

Fatty Acides	*Mejhoul*	*lkhalt*	*Boufkouss*	*Iklan*	*Bouslikhen*	*Bousthammi*	*Ablouh*	*Jihel*	*Aziza*	*Lkenz*
Saturated fatty acid										
C8	0.35	0.23	0.24	0.13	0.02	0.27	0.22	0.35	0.34	0.28
C10	0.40	0.35	0.31	0.20	0.03	0.41	0.44	0.45	0.43	0.32
C12	19.39	19.90	18.88	15.90	15.62	23.00	22.80	20.76	19.51	17.69
C14	10.49	11.34	10.14	10.47	8.63	12.70	12.31	11.20	9.93	9.95
C16	9.87	10.95	9.64	10.33	10.47	10.19	10.15	10.73	10.49	9.37
C17	0.07	0.08	0.06	0.07	0.07	0..05	0.06	0.07	0.09	0.05
C18	4.09	3.67	3.59	3.30	3.33	3.72	3.64	4.21	3.88	3.80
C20	0.47	0.37	0.52	0.41	0.54	0.54	0.46	0.64	0.47	0.40
Total of SFA	45.13	46.89	43.38	40.81	38.71	50.83	50.08	48.41	45.14	41.86
Monounsaturated fatty acid										
C16:1	0.16	0.16	0.11	0.12	0.12	0.09	0.09	0.10	0.09	0.07
C17:1	0.03	0.03	0.04	0.04	0.04	0.03	0.03	0.03	0.05	0.03
C18:1	45.45	44.83	47.23	50.27	52.60	42.13	42.43	45.23	45.58	45.70
C20:1	0.28	0.28	0.45	0.44	0.49	0.43	0.39	0.42	0.44	0.28
Total of MUFA	45.92	45.30	47.83	50.87	53.25	42.68	42.94	45.78	46.16	46.08
Polyunsaturated fatty acid										
C18:2	8.87	7.61	8.59	8.12	7.45	6.18	6.84	5.76	8.33	9.27
C18:3		0.11	0.07	0.08	0.07	0.03	0.03	0.04	0.06	0.06
Total of PUFA	8.87	7.72	8.66	8.20	7.52	6.21	6.87	5.80	8.39	9.33

*SFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: Polyunsaturated fatty acid.

In the kinds of saturated fatty acids, the total SFA was from 38.71% to 50.83%, while the predominant SFA was capric acid (C12:0), with the highest value of 23% recorded in Bousthammi's variety. Followed by palmitic (C16:0) and myristic (C14:0) with a similar average value of 10.22% and 10.72% respectively. The second group of the fatty acids was the monosaturated fatty acids; the total of MUFA was slightly higher than the total of SFA ranging from 42.68% to 53.25%. The oleic acid (C18:1) recorded the highest value of the group with 52.60% and 42.13% for Bouslikhen and Bousthammi respectively. Then there were the Gadoleic (C20:1), Palmitoleic (C16:1), and Margaroleic (C17:1) acids, which had the lowest average values of 0.39%, 0.11%, and 0.03%, respectively.

Compared to the first and second groups, the final group showed the lowest value of the total PUFA ranging from 5.8% to 9.33%. Also, this group contains only two fatty acids, the highest percentage was recorded in linoleic acid (C18:2) from 5.76% to 9.27%. While the linolenic acid (C18:3) showed the lowest value ranged between 0.03% and 0.11%.

According to these results, the DSO of Bouslikhen's variety is rich in unsaturated fatty acids with MUFA+PUFA= 60.77% while the lowest was for Bousthammi with MUFA + PUFA = 48.89%. Conversely, DSO of Bousthammi's variety contains the highest amount of saturated fatty acid with an SFA = 50.83% whereas DSO of Bouslikhen recorded the poorest value of SFA=38.71%.

For the first and second groups of fatty acids, the highest values were recorded for the oleic and lauric acid. According to the literature, when the predominant acid is oleic acid followed by lauric acid, DSO is considered as oleic-lauric type oil. For example, in Iran DSO is also considered an oleic-lauric type (**Dehdivan & Panahi, 2017**). Omani DSO was stated as an oleic-myristic type (**Suresh et al., 2013**), while DSO of United Arab Emirate was reported as an oleic-linoleic or oleic-linolenic type (**Al-Hooti et al., 1997**).

DSO's composition of sterols

The total sterol content of all DSO varieties can be divided into 3 groups (Table 3). The first one has a total amount under 400 mg/100 g of oil which include the variety of Lkenz with 346.97 mg/100 g of oil, Ablouh with 369.9 mg/100 g of oil, and Jihel with 371.65 mg/100 g of oil. Then comes the second group under 500 mg/100 g of oil, comprising five varieties such as Mejhoul (414.58 mg/100g of oil), lkhalt (422.57 mg/100g of oil), Bousthammi (432.85 mg/100 g of oil), Bouslikhen (484.13 mg/100 g of oil), and Aziza (495.79 mg/100 g of oil). The last group contains two varieties with a total sterol content that exceeds 500 mg/100 g of oil; this is the case of Iklan and Boufkouss varieties with 552.69 mg/100 g of oil and 816.60 mg/100 g of oil. According to the literature results, the amount of sterols found in DSO is similar to other seed oils, such as *P.canariensis* seed oil (336 mg/100 g oil) (**I. Nehdi et al., 2010**), rapeseed (500 mg/100 g oil), soybean (900 mg/100 g oil) (**Sabir et al., 2003**), and date pit oil (350 mg/100 g oil) (**Besbes et al., 2004**).

The phytosterols are usually found in the esterified forms in oils (**Christie & Han, 2012**). The determination of these phytochemical compounds serves to analyze the quality of the oil and detect commercial frauds. They are stated as the unsaponifiable fraction of the major components (**Lercker & Rodriguez-Estrada, 2000**). Eight sterols (cholesterol, brassecosterol, campesterol, stigmasterol, β-sitosterol, Δ5-avenasterol, Δ7-stigmasterol and Δ7-avenasterol) were identified in DSO. The β-sitosterol recorded the highest level in each variety of DSO and consisted of 30.79-69.71% of the total detected sterols. A similar observation was reported in several studies (**Besbes et al., 2004**)(**I. Nehdi et al., 2010**).

Then Δ5-avenasterol, campesterol, Δ7-stigmasterol, and stigmasterol came right over the predominant. The average value ranged from 12.76% to 4.07%. Finally, the lower concentration of sterols was registered for brassecosterol, cholesterol, and Δ7-avenasterol with 1.44%, 1.00%, and 0.41% respectively.

β-sitosterol is an important phytosterol in the human diet. It is synthesized by melonic acid in plants. It has a wide range of pharmaceutical activities, including antipyretic, antacid, angiogenic, antioxidant, immunomodulatory, antihyperlipidemic, anti atherosclerotic, and cholesterol levels. It also affects the central nervous system, liver function, gastrointestinal tract, and reproductive system, which may be the basis of its use for the treatment of diseases or disorders of this system (**Bin Sayeed et al., 2016**).

Table 3 Sterolic composition (%) of ten varieties of DSO.

Sterols	*Mejhoul*	*lkhalt*	*Boufkouss*	*Iklan*	*Bouslikhen*	*Bousthammi*	*Ablouh*	*Jihel*	*Aziza*	*Lkenz*
Cholesterol	0.9±0.01^{a}	1.73±0.02^{b}	0.89±0.01^{a}	2.05±0.05^{c}	1.70±0.02^{b}	1.12±0.02^{d}	0.81±0.01^{a}	1.42±0.02^{e}	2.7±0.03^{f}	1.05±0.02^{d}
Brassecosterol	1.32±0.2^{a}	1.13±0.13^{abe}	0.52±0.02^{c}	0.71±0.01^{ce}	0.96±0.03^{ae}	1.17±0.03^{af}	1.22±0.02^{a}	1.32±0.01^{a}	0.77±0.02^{bcf}	0.9±0.04^{ac}
Campesterol	7.34±0.30^{a}	8.87±0.10^{b}	4.84±0.11^{c}	9.53±0.30^{bd}	13.48±0.21^{e}	9.37±0.22^{bf}	11.82±0.10^{g}	11.25±0.20^{gi}	10.33±0.30^{dfi}	8.64±0.31^{b}
Stigmasterol	4.56±0.20^{ae}	4.53±0.13^{ae}	2.42±0.30^{b}	4.25±0.20^{af}	4.23±0.13^{ae}	3.07±0.07^{bd}	3.57±0.21^{ac}	3.54±0.10^{acd}	5.01±0.01^{ef}	5.47±0.30^{e}
Beta-sitosterol	55.48±2.03^{a}	56.24±1.5^{a}	30.79±1.00^{b}	69.71±1.21^{c}	65.32±2.00^{c}	63.13±1.23^{ac}	66.10±2.27^{c}	62.96±1.48^{ac}	62.76±1.23^{ac}	67.11±2.04^{c}
delta-5-avenosterol	9.78±1.28^{a}	11.91±2.03^{b}	16.35±1.32^{c}	10.23±1.78^{a}	10.01±2.21^{a}	16.83±1.38^{d}	12.50±1.01^{e}	15.31±1.73^{f}	11.79±2.11.40^{b}	12.92±1.39^{e}
delta-7-Stigmasterol	15.20±1.23^{a}	12.19±1.72^{b}	41.30±2.04^{c}	0.18±0.02^{df}	0.47±0.09^{d}	1.81±2.04^{e}	0.34±0.02^{df}	0.33±0.01^{d}	1.81±0.08^{eg}	0.14±0.01^{fg}
delta-7-avenosterol	0.18±0.02^{a}	0.56±0.08^{b}	0.12±0.01^{c}	0.34±0.04^{d}	0.63±0.07^{e}	0.49±0.02^{f}	0.60±0.01^{be}	0.30±0.02^{d}	0.55±0.04^{b}	0.34±0.02^{d}
Total sterol mg/100g of oil	414.58±2.49^{a}	422.57±3.29^{b}	816.60±2.19^{c}	552.69±3.27^{d}	484.13±2.14^{e}	432.85±3.2^{f}	369.9±2.21^{g}	371.65±2.46^{g}	495.79±1.93^{h}	346.97±2.4^{i}

The results are expressed in the form of anaverage of 2 individual repetitions (n = 2e ± SEM). The mean followed by similar letters exposing in the column are not different (P < 0.05).

DSO's composition of tocols

The analysis of tocol compositions revealed that DSO contained a high amount of α-, γ-tocopherol abbreviated as AT, and GT, respectively and α-, γ-tocotrienol denoted as AT3, GT3, respectively in Table 4 with the total amount of tocols.

DSO contains an important amount of vitamin E isomers. The total of tocols higher than 3000 µg/g was registered for the varieties Boufkouss, Mejhoul, Aziza, and Jihel. Then the varieties between 3000 µg/g and 2000 µg/g of total tocols were Lkhalt, Iklan, Lkenz, Bousthammi, and Bouslikhen. At last, there was only the variety of Ablouh below 2000 µg/g of total tocols.

The predominant tocol was AT3 (1406.66-596.88 µg/g of oil) for lkhalt, bouslikhen, bousthammi, and aziza while the predominant for mejhoul, boufkouss, iklan, ablouh, jihel, and lkenz was AT (1584.57-248.17 µg/g of oil) followed by GT3 ranging from 456.05 µg/g of oil to 37.85 µg/g of oil and GT from 272.92 µg/g of oil to 12.89 µg/g of oil respectively for all varieties. According to Nehdi et al. results, the variety of Bahri had a similar value to our variety of Lkenz, with GT 289.2 µg/g of oil and 272.92 µg/g of oil respectively, then GT3 with 318.8 µg/g of oil and 318.54 µg/g of oil. Unlike to Al Juhaimi et al. study, they recorded a lower value of GT with 82.6 µg/g of oil and GT3 with 118.4 µg/g of oil. There is a significant difference in the content of tocopherols and tocotrienols depending on variety, maturity, and location. Tococpherols are well known to have a strong antioxidant capacity, like vitamin E who offers a good protection of body against oxidation reactions or radicals that carry cholesterol and lipoproteins to membranes. It also reacts as a screening reagent to prevent UV-induced skin damage and aging, suppress cancer cell growth, and act as a protective agent against cirrhosis by lowering cholesterol levels (**I. A. Nehdi et al., 2018**); (**Al Juhaimi et al., 2018**).

Table 4 Tocols composition (µg/g) of DSO.

Tocol	AT	AT3	GT	GT3	Total tocol µg/g of oil
Mejhoul	279.38±1.00^{a}	33.99±1.00^{a}	14.26±1.05^{a}	46.56±1.00^{a}	3686.27±2.00^{a}
lkhalt	309.19±0.10^{b}	1406.66±2.02^{b}	105.57±2.00^{b}	432.12±2.02^{b}	2650.29±1.10^{b}
Boufkouss	248.17±1.12^{c}	29.39±0.30^{a}	12.89±0.9^{a}	37.85±1.10^{a}	3844.06±1.13^{c}
Iklan	622.60±0.3^{d}	547.42±1.1^{c}	27.7±0.60^{c}	78.10±1.12^{c}	2539.31±1.20^{d}
Bouslikhen	553.10±1.70^{e}	737.99±1.00^{d}	167.25±2.10^{d}	335.67±2.11^{d}	2079.56±1.32^{e}
Bousthammi	561.81±1.14^{f}	596.88±1.99^{e}	154.13±1.00^{e}	456.05±2.05^{e}	2157.06±2.06^{f}
Ablouh	522.36±2.00^{g}	509.81±0.11^{f}	147.66±1.12^{e}	392.66±1.13^{f}	1937.14±1.04^{g}
Jihel	786.3±1.11^{k}	740.12±2.02^{d}	36.75±1.17^{f}	105.93±2.03^{g}	3165.97±2.02^{h}
Aziza	477.17±1.10^{l}	914.01±1.00^{g}	45.60±1.10^{g}	97.79±1.12^{g}	3486.64±2.04^{k}
Lkenz	1584.57±1.12^{m}	30.51±1.20^{a}	272.92±2.02^{h}	318.54±2.3^{h}	2275.63±0.97^{l}

The results are expressed in the form of anaverage of 2 individual repetitions (n = 2e ± SEM). The mean followed by similar letters exposing in the column are not different (P < 0.05).

Statistical analysis

Correlation Matrix

Table 5 shows the correlation coefficients of the various physicochemical indicators of oil quality (acid value, Saponification Value, K232, K270, Iodine Value, Peroxide Value), total sterols, total tocols, chlorophyll, carotenoid, linoleic (C18:2) and linolenic (C18:3). In addition, Table 6 shows the correlation matrix coefficient p-values.

Table 5 Pearson's correlation matrix coefficient between different parameters used.

Variables	Total sterols	Total tocols	Carotenoids	Chlorophylls	Linoleic C18:2	Linolenic C18:3	IV	FFA%	PV	SV	K232	K270
Total sterols	**1**	0.508	0.251	0.273	0.304	0.322	0.439	0.259	0.456	-0.627	-0.264	-0.236
Total tocols	0.508	**1**	-0.116	-0.115	0.361	-0.130	0.195	0.130	0.426	-0.294	-0.598	-0.242
Carotenoids	0.251	-0.116	**1**	**0.979**	-0.565	0.034	-0.339	0.303	-0.305	-0.061	0.327	-0.024
Chlorophylls	0.273	-0.115	**0.979**	**1**	-0.579	0.036	-0.401	0.382	-0.245	-0.022	0.378	0.067
Linoleic C18:2	0.304	0.361	-0.565	-0.579	**1**	0.156	**0.736**	-0.046	**0.681**	-0.198	-0.224	-0.034
Linolenic C18:3	0.322	-0.130	0.034	0.036	0.156	**1**	0.402	-0.103	0.588	-0.279	0.211	0.023
IV	0.439	0.195	-0.339	-0.401	**0.736**	0.402	**1**	-0.018	0.610	-0.212	0.046	-0.103
FFA%	0.259	0.130	0.303	0.382	-0.046	-0.103	-0.018	**1**	0.300	0.520	0.215	0.242
PV	0.456	0.426	-0.305	-0.245	**0.681**	0.588	0.610	0.300	**1**	-0.066	0.017	0.067
SV	-0.627	-0.294	-0.061	-0.022	-0.198	-0.279	-0.212	0.520	-0.066	**1**	0.507	0.598
K232	-0.264	-0.598	0.327	0.378	-0.224	0.211	0.046	0.215	0.017	0.507	**1**	0.601
K270	-0.236	-0.242	-0.024	0.067	-0.034	0.023	-0.103	0.242	0.067	0.598	0.601	**1**

Table 6 p-values of the correlation matrix coefficient.

Variables	Total sterols	Total tocols	Carotenoids	Chlorophylls	Linoleic C18:2	Linolenic C18:3	IV	FFA%	PV	SV	K232	K270
Total sterols	**0**	0.134	0.485	0.446	0.392	0.364	0.204	0.470	0.185	0.052	0.461	0.512
Total tocols	0.134	**0**	0.750	0.752	0.305	0.721	0.589	0.720	0.220	0.410	0.068	0.500
Carotenoids	0.485	0.750	**0**	**< 0.0001**	0.089	0.925	0.339	0.394	0.392	0.868	0.356	0.947
Chlorophylls	0.446	0.752	**< 0.0001**	**0**	0.079	0.920	0.251	0.276	0.495	0.952	0.282	0.854
Linoleic C18:2	0.392	0.305	0.089	0.079	**0**	0.667	**0.015**	0.899	**0.030**	0.583	0.533	0.926
Linolenic C18:3	0.364	0.721	0.925	0.920	0.667	**0**	0.250	0.777	0.074	0.435	0.559	0.949
IV	0.204	0.589	0.339	0.251	**0.015**	0.250	**0**	0.961	0.061	0.557	0.900	0.777
FFA%	0.470	0.720	0.394	0.276	0.899	0.777	0.961	**0**	0.400	0.123	0.550	0.500
PV	0.185	0.220	0.392	0.495	**0.030**	0.074	0.061	0.400	**0**	0.856	0.964	0.855
SV	0.052	0.410	0.868	0.952	0.583	0.435	0.557	0.123	0.856	**0**	0.135	0.068
K232	0.461	0.068	0.356	0.282	0.533	0.559	0.900	0.550	0.964	0.135	**0**	0.066
K270	0.512	0.500	0.947	0.854	0.926	0.949	0.777	0.500	0.855	0.068	0.066	**0**

Table 5 shows the Pearson correlation, which was used to examine the connections between the various factors examined in this research. Table 6 also shows the p-values of the correlation matrix coefficients for all variables. As a result, we found a substantial positive connection (p-value < 0.0001) between chlorophyll and carotenoid (r^2 = 0.979). Furthermore, with r^2 = 0.736, there was a positive connection (p-value < 0.05) between linoleic (C18:2) and the Iodine Value. linoleic (C18:2) and Peroxide Value (r^2 = 0.681) also had a strong positive connection (p-value < 0.05).

Principal Component Analysis (PCA)

Total sterol, total tocopherol, chlorophyll, carotenoid, linoleic acid (C18:2), linolenic acid (C18:3), and oil quality characteristics are all considered variables. The PCA uses the F1-F2 factorial design to project them (Figure 1). The first main component (F1) accounts for 31.92 percent of the total data, while the second main component (F2) accounts for 20.47 percent. The linear combination is representative of the variables since the cumulative proportion of the two initial main components is more than 50% (52.39 percent). In reality, the first two axes are adequate for representing the whole data set. The plane created by axes F1 and F2 in Figure 2 depicts the correlation between the variables. The positive connection between total tocopherol, chlorophyll, carotenoid, K270, and K232 forms the foundation of the F1 axis. The positive connection between linoleic (C18:2), peroxide, and Iodine Value forms Axis F2.

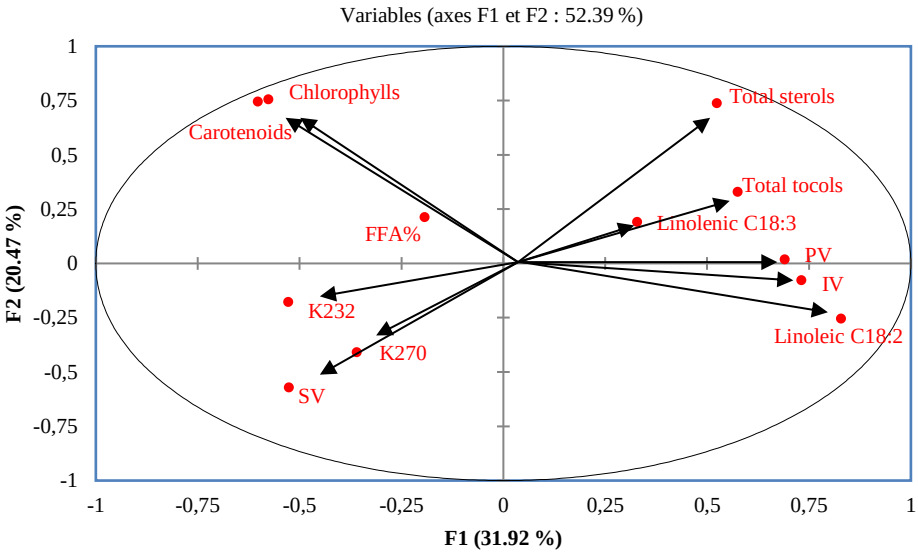

Figure 1 PCA factorial plan was used to analyze the values of several physicochemical properties of various DSO samples.

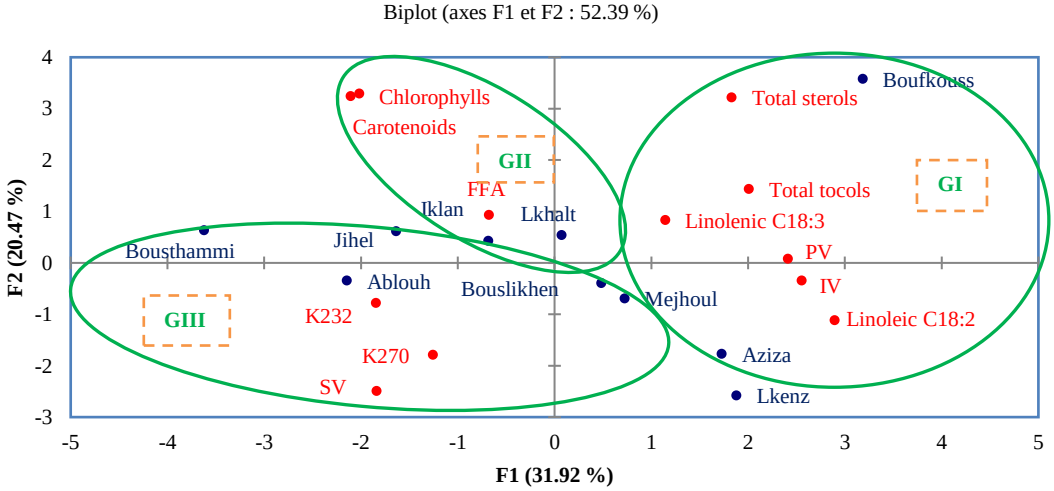

Figure 2 Individual projection on the factorial scheme (F1F2). GI stands for Group I, GII for Group II, and GIII for Group III.

The projection of our variables onto the factorial plan (F1-F2) resulted in their being split into three distinctive groups, as illustrated in Figure 2. Boufkouss, Lkenz, Mejhoul, and Aziza are the four types that comprise Group I. They have a high concentration of total tocol, total sterol, linoleic C18:2, and linolenic acid C18:3, as well as a high iodine and peroxide value. They did, however, have lower levels of carotenoid and chlorophyll than Group III. Iklan and Lkhlat belong to Group II, and they have the greatest specific UV extinction coefficient (K232 and K270), FFA percent, and saponification value. But also, they had an average content of carotenoids and chlorophyll. The last group (Group III) has four species: Bouslikhen, Ablouh, Jihel, and Bousthammi, with a high content of carotenoid and chlorophyll. Their oil recorded the lowest values of the physicochemical parameters of oil quality. On other hand, the total of sterol and tocol is lower than Group I.

Hierarchical clustering analysis (HCA)

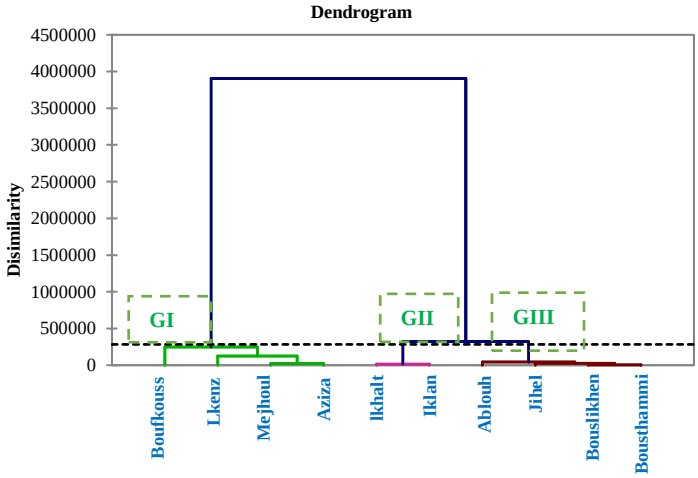

Figure 3 Dendrogram of the sample's oils studied obtained by cluster analysis (Ward and Euclidean distance).

In order to assess the similarity measure, 10 variants of DSO were categorized using the Wards technique and the squared Euclidean, as shown in the dendrogram in Figure 3. HCA is used to evaluate the correlation between all samples, and then to illustrate their similarities using data from physicochemical characteristics of oil quality and chemical composition of oil, such as total sterol, total tocol, chlorophyll, carotenoid, linoleic (C18:2) and linolenic (C18:3).

Ten varieties of DSO have been divided into three clusters. Cluster I contains four varieties of DSO (Boufkouss, Lkenz, Mejhoul, and Aziza). They account for 40% of the total. They are characterized by a high mean value of total sterol with 524.656 mg/100 g oil (Table 7), total tocol with 3545.737 µg/g of oil, linoleic acid C18:2 with 7.888%, and Iodine Value of 0.553 g I_2/100 g. But compared to other clusters data, they have a lower mean value of carotenoid (5.257 mg/kg) and chlorophyll (11.513 mg/kg). Cluster II is only formed by Iklan and Lkhlat and it has a high mean value of linolenic (C18:3) of 0.095%. They also recorded the highest parameters of oil quality; the FFA was 2.26%, and the mean saponification value was 263.195 mg KOH/g oil, the same for the specific UV extinction coefficients with 2.230 in K232 and 1.840 in K270 (Table 7). Cluster III represents 40% and contains four DSO varieties named Bouslikhen, Bousthammi, Ablouh, and Jihel respectively. This cluster is characterized by the highest mean values of chlorophyll (20.81 mg/kg), carotenoid (7.95 mg/kg), and a medium mean value of FFA (2.37%), and saponification value (263.55 mg KOH/g oil). These results are in agreement with the data from the PCA, where the distribution of all extracts on the score plot indicates a similar trend. Furthermore, the PCA results were consistent with those of HCA.

Table 7 Parameter's data of each cluster.

Class	Total sterols (mg/100 g oil)	Total tocols (µg/g of oil)	Carotenoids (mg/kg)	Chlorophylls (mg/kg)	Linoleic C18:2 (%)	Linolenic C18:3 (%)	IV (g I_2/100 g oil)	FFA%	PV (meq O2/kg)	SV (mg KOH/g oil)	K232	K270
1	524.656	3545.737	5.257	11.513	7.888	0.043	0.553	2.165	5.330	229.465	1.564	1.363
2	487.630	2594.797	7.238	16.533	7.865	0.095	0.569	2.350	5.930	263.195	2.230	1.840
3	408.463	2112.348	5.345	11.945	7.435	0.048	0.544	2.010	4.698	245.405	1.988	1.515

CONCLUSION

This study reveals that DSO is a valuable source of neutraceutical and edible oil. Due to its high content of oleic and lauric acid, DSO is considered an oleic-lauric type that has a preventive action on cardiovascular illnesses. DSO recorded the highest SFA in the Ablouh and Bousthammi varieties with 50.08 and 50.83%, then Iklan and Bouslikhen registered the main values of oleic acid (50.27-52.60%) and MUFA (50.87-53.25%), while Boufkouss and Mejhoul showed a greater amount of PUFA (8.66-8.87%). DSO contains a significant amount of sterol (369.9–816.60 mg/100g of oil), and all varieties contain a significant amount of β-sitosterol particularly Lkenz variety (64.11 mg/100g of oil). Meanwhile, this by-product also possesses a rich source of tocol, ranging from 1937.14 µg/g of oil (Ablouh) to 3844.06 µg/g of oil (Boufkouss). The analysis of the physicochemical parameters of acid value (AV), peroxide value (PV), saponification value (SV), iodine value (IV), K232, and K270 shows that Lkenz variety recorded the lowest values of FFA (1.10%), SV (214.85mg KOH/g oil), and chlorophyll (3.44mg/kg). Boufkouss had the lowest coefficients of K232 and 270 with 1.28 and 0.78, respectively. Similarly, the lowest carotenoid, PV, and IV levels were 2.21 mg/kg (Aziza), 3.88 meq O_2/kg (Jihel), and 48.84 g I_2/100g oil (Bousthammi). In contrast, Bousthammi showed the highest values of chlorophylls (20.81 mg/kg), SV (225.59 mg KOH/g oil), K232 (2.42), and carotenoids (7.95 mg/kg). This examination indicates that DSO has high oxidative stability (can be easily conserved), can be considered as a non-drying oil, and may be used as a substitute for some conventional oils. According to these and several other results, date seed oil has a potential application in several cosmetic formulations, such as body creams, shaving soaps and shampoos, and pharmaceutical products. However, more tests of safety should be conducted before it is used by the factories that make food and cosmetics.

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