

Identification of Date Seeds Varieties Patterns to Optimize Nutritional Benefits of Date Seeds

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Abstract

Bioactive compounds, like fibers and polyphenols, of plant products are recognized for their health benefits. A large amount of these bioactive compounds can be found in date seeds. The health benefit characteristic of such bioactive compounds depends on the type of food they are found in. However, further research is needed in examining how these compounds interact to give them these beneficial health effects.

The aim of this study was to describe the macro, micro nutrients and polyphenols content, color and antioxidant properties of eighteen varieties of date seeds, and to examine how these parameters interact and identify patterns of varieties.

Factorial analysis was performed to study the interrelations between these parameters and identify date seeds patterns.

Overall, date seeds showed a great nutritional value, highlighting their enormous potential as a functional ingredient. Different patterns of nutritional, antioxidant and color parameters were identified, suggesting that the health benefit may vary according to the pattern of the date seeds variety.

Keywords: Date seeds; Antioxidant capacity; Patterns; Antioxidant compounds; Nutritional composition; Color

Introduction

Date seeds, which constitute 6-15% of the total weight of the ripe date, are largely produced in the Middle East and especially in the United Arab Emirates (UAE). In 2004, the world production of date seeds reached approximately 863 thousand tons. Although, a high nutritional value is well-recognized for date seeds they are being wasted in large quantities or used for animal (camels, cattle, sheep, and poultry) feed.

The nutritional value of date seeds is mainly related to a significant amount of dietary fiber and a high content of polyphenols [1,2]. Dietary fibers are particularly well-known for their potential benefits on cancer and type 2 diabetes prevention [3]. Polyphenols, including chemical compounds like phenolic and flavonoids, are common constituents of the human diet, with fruits and vegetables being the major dietary sources of these bioactive compounds. Their possible health benefits have been suggested to derive from their antioxidant properties by chelating redox-active metal ions, inactivating lipid free radical chain reactions, preventing hydro peroxide conversion into reactive oxradicals, and from their anti-inflammatory properties [4].

The current environment and trend to move toward a westernized diet and a sedentary lifestyle, have been associated with an overproduction of free radicals and reactive oxygen species (ROS) or insufficient ROS detoxification, contributing to oxidative stress in human. Oxidative stress is defined as an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily neutralize these compounds using antioxidant systems, leading to cell damages and cellular mechanisms disruptions. This has been associated to the development of cardiovascular diseases, obesity, diabetes and cancer. This emphasizes the importance of all natural antioxidants, including polyphenols, which are likely to strengthen the oxidative system in human body and prevent the development of chronic diseases, and contribute to health promotion [5].

The promising role of polyphenols is reinforced by experimental studies on animals and human cell lines, which demonstrated that they can contribute to the prevention of degenerative diseases, cancer, cardiovascular diseases and obesity as well [6,7].

Nonetheless, the nature and the intensity of the effects of polyphenols are likely to vary according to the chemical type of the polyphenol compound. Indeed, flavan-3-ols, including catechin and epicatechin which were the most abundant polyphenols detected in date seeds extract from Khalas variety, have been described in literature as having the highest beneficial health effects among the polyphenols. It has been suggested that the molecular target and the activated signaling pathway could vary from a polyphenol compound to another one, resulting to different protective effects [8].

One factor that is that is likely to affect the polyphenols' profile in plant products and more generally the nutrient's composition, is the cultivar. Indeed, since significant differences in nutritional and antioxidant properties between varieties have been commonly reported in other plant seeds [7], similar varietal differences can be expected in date seeds. In regards to the date palm, there are more than 100 known cultivars.

According to the variety, the polyphenolic profile, the nutritional

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properties, and the antioxidant properties, may differ and potentially result in different health impacts. In other words, this suggests that the interest of date seeds would depend on the variety of the seed and on the targeted health benefit. Overall, the field of varietal differences in the date seeds needs to be further studied.

Due to its high polyphenols content and nutritional value, date seeds represent a potential functional agent in the food industry, cosmetics and pharmaceuticals [9]. However, further research is needed to determine the specificities in terms of nutritional and antioxidant properties of each variety of date seed. This is due to the differences of the nutrients and polyphenols' composition on antioxidant properties.

The objective of this research was to describe the macro, micro nutrients and polyphenols content, color and antioxidant properties of eighteen varieties of date seeds, and to identify the specificities of each variety.

Materials and Methods

Material

Date palm seeds of the following varieties; Khalas, Barhe, Lulu, Shikat alkahlas, Sokkery, Bomaan, Sagay, Shishi, Maghool, Sultana, Fard, Maktoomi, Naptit saif, Jabri, Kodary, Dabbas, Raziz, and Shabebe, were used in this study. All 18 varieties were obtained at the "tamr" stage (fully ripe dates), from a well-known commercial date pack house in the UAE (Al Ain Dates Factory) and were subjected to uniform harvest and post-harvest treatments [10]. The summer season is when the tamr is collected, and it is usually spread over a period of 2–3 months, i.e. the industry receives freshly harvested tamr batches over a 2–3-month period. With no preference to size, color, appearance or firmness, 5 kg from each of the seed varieties were collected randomly from tamr batches at the end of the season and were analyzed. After collection, the seeds were soaked in water, washed to get rid of any adhering date flesh, and then air-dried. Date seeds of each variety were separately ground to powder form in a heavy-duty grinder (IKA M 20 Universal Mill; IKA werke GmbH Co. KG, Staufen, Germany), and each sample was estimated in triplicate.

Methods

Extraction: The extraction of antioxidant compounds from 18 date seed varieties was carried out using methanol/H₂O (50:50, v/v). The date palm seed variety sample (1 g) was extracted using 40 mL methanol/H₂O (50:50, v/v).

Nutritional composition

Protein content: Total protein was determined by the Kjeldahl method. Protein was calculated using the general factor (6.25) [11].

Fat content: Fat was measured by extracting with light petroleum ether and then removing the solvent by distillation. The residue was dried at 103°C and the fat content determined gravimetrically.

Dietary fiber: Determination of dietary fiber was carried out using the AOAC enzymatic gravimetric official method (Method 991.43) [12].

Micronutrients composition: The levels in different minerals including Calcium (Ca), Phosphorus (P), Potassium (K), Magnesium (Mg), Iron (Fe), and zinc (Zn) were measured.

Samples were prepared for the determination of minerals as described by Heckman [13]. The minerals were determined using

inductively coupled plasma atomic emission spectrometry (Varian-Vista-MPX; Varian, Inc. Palo Alto, CA, USA) as outlined in the manufacturer's manual.

Antioxidant properties

Total phenolics content: Total phenolics were evaluated using the spectrophotometric analysis with Folin–Ciocalteu's phenol reagent according to Kim [14]. The standard curve for total phenolics was made using gallic acid standard solution (0–100 mg/L) and total phenolics were expressed as mg of gallic acid equivalent (GAE)/100 g of date seed.

Total flavonoids content: Total flavonoids were determined using the method of Zhishen [15]. An aliquot (250 µl) of each extract or standard solution was mixed with 1.25 ml of H₂O and 75 µl of 5% NaNO₂ solution. After 6 min, 150 µl of 10% AlCl₃ solution was added. After 5 min, 0.5 ml of 1 M NaOH solution was added and then the total volume was made up to 2.5 ml with H₂O. The absorbance against blank was read at 510 nm. The results were expressed in terms of mg rutin equivalent (RE)/100 g date seed.

(+)-Catechin and (-)-Epicatechin contents: Determination (+)-catechin and (-)-epicatechin in date seeds extracts was performed according to the method of Satoh [16]. In brief, high-performance liquid chromatography equipped with 125 binary HPLC pump, 2475 fluorescence detector, 717 plus auto sampler and inline degasser AD was used (Waters Corporation, Milford, MA., USA). Reverse-phase separation was carried out using symmetry C18, 5 µm, and 4.6x150 mm column, equipped with a C18 precolumn. An 15-min isocratic elution was performed using mobile phase of 9% acetonitrile and 2% acetic acid with flow rate of 1ml/min. Detection was carried out using fluorescence detector set at λEX= 280nm and λEM= 315 nm. Catechin and epicatechin were quantified by external standard method. Plotting concentration (µg/mL) to peak area was set up by the calibration graphs.

Total antioxidant capacity: The total antioxidant capacity (TAC) of date seeds extracts was investigated according to the method of Prieto [17]. In brief, 0.1 ml of date seed extract was mixed with 0.3 ml of the reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate and 4 mM ammonium molybdate). The tubes were capped and the reaction mixture then incubated for 90 min at 95°C. The absorbance of the cooled mixture was measured at 695 nm against a blank. The blank contained the reagent solution and solvent. TAC was expressed as the absorbance of the sample. The higher absorbance value indicated higher antioxidant activity.

Superoxide radical (O₂⁻) scavenging assay: Superoxide anion (O₂⁻), the one-electron reduced form of molecular oxygen, is one of the most representative free radicals. Superoxide anion is a precursor to active free radicals that have the potential of reacting with biological macromolecules, and thereby inducing tissue damage [18,19]. In cellular oxidation reactions, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of cell-damaging free radicals and oxidizing agents.

Superoxide radical scavenging assay was determined as per the method described by Gao and Marklund [20,21]. O₂⁻ was generated from autoxidation reaction of pyrogallol. Eighty microliters of date seed extract was mixed with 80 µl of 50 mM Tris–HCl buffer (pH 8.3) containing 1 mM EDTA in a 96-well microplate followed by the addition of 40 µl of 1.5 mM pyrogallol in 10 mM HCl. The rate of O₂⁻-induced polymerisation of pyrogallol (ΔA/min s) was measured as

increase in absorbance at 420 nm for 4 min at room temperature. Tris-HCl buffer was used instead of seed extract as blank ($\Delta A/\text{min c}$). All assays were performed in triplicate, and the scavenging activity of the seed extract was calculated using

$$\text{Activity} = [(\Delta A/\text{min c} - \Delta A/\text{min s}) / \Delta A/\text{min c}] \times 100\%$$

Ferric-reducing antioxidant power assay (FRAP): FRAP assay depends on the reduction of ferric tripyridyltriazine (Fe (III)-TPTZ) complex to the ferrous tripyridyltriazine (Fe (II)-TPTZ) by a reductant (antioxidants or other reducing agents) at low pH. Fe (II)-TPTZ has an intensive blue color and can be monitored at 593 nm. FRAP was performed according to the method of Benzie and Strain [22] with slight modifications. The FRAP reagent included 10 mM TPTZ solution in 40 mM HCl, 20 mM FeCl₃ solution and 0.3 M acetate buffer (pH 3.6) in proportions of 1:1:10(v/v/v). 50 μ L of diluted methanolic extracts were mixed with 3 mL of freshly prepared FRAP reagent and the reaction mixtures incubated at 37 °C for 30 min. Absorbance was determined at 593 nm against distilled water blank. Aqueous solutions of ferrous sulfate (100–2000 μ M) were used for calibration. Triplicate measurements were taken and the FRAP values were expressed as μ mol of ferrous equivalent (FE)/100 g of date seed.

DPPH free radical-scavenging assay: The antioxidant activities of the seeds extracts were also studied through the evaluation of the free radical-scavenging effect on the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical. DPPH is a free radical and stable at room temperature, which produces a violet solution in ethanol. Reduction of DPPH by antioxidants results in a loss of absorbance. Thus, the degree of discoloration of the solution indicates the scavenging efficiency of the added substances. The use of DPPH provided an easy and rapid way to evaluate antioxidant activity.

The determination was based on the method proposed by De Ancos [23]. An aliquot (10 μ L) of seed extract was mixed with 90 μ L of distilled water and 3.9 ml of 0.25 mM DPPH in methanol. The mixture was thoroughly vortex-mixed and kept in the dark for 30 min. The absorbance was later measured at 515 nm, against a blank of methanol without DPPH. Results were expressed as percentage of inhibition of the DPPH radical. Percentage of inhibition of the DPPH radical was calculated according to the following equation:

$$\% \text{ inhibition of DPPH} = [(\text{Abs control} - \text{Abs sample}) / \text{Abs control}] \times 100 \text{ where Abs control is the absorbance of DPPH solution without extracts.}$$

Color: The color of date seeds sample was measured with a TC-PIIG automatic color difference meter using a Hunter Lab model (ColorFex; Hunter Associates Laboratory Inc., USA). Color measurements were expressed as tristimulus parameters, L*, a*, and b*. L* indicates lightness (100= white and 0 =black). a* indicates redness–greenness and b* indicates yellowness–blueness [24].

Statistical Analysis: Statistical analysis was performed using SPSS for windows (version 20; SPSS Inc., Chicago, Illinois, USA).

Univariate analysis: All analytical determinations were performed in triplicate. Means \pm standard deviation were calculated. Correlation coefficients were calculated to study the relationship between nutritional characteristics, antioxidant properties, and color parameters of the eighteen varieties of date seeds. Spearman coefficient was used. The statistical significance was set at $p < 0.05$.

Multivariate analysis: Factor analysis was used to identify major

patterns of date seeds varieties based on their nutritional characteristics (protein, dietary fiber and fat contents), antioxidant properties (total phenolics, total polyphenols, catechin and epicatechin levels, FRAP, DDPH, Superoxide inhibition and TAC) and color parameters (L, A and B) and some nutritional characteristics (protein, fiber and fat contents).

Principal Component Analysis (PCA) was done with the factors rotated by orthogonal transformation. The purpose of this method is to determine the axes or planes that provide the most informative graphical representation of the relationships between the variables listed above. The ability of an axis to describe the layout is quantified by the percentage of information represented by this axis and by its eigenvalue. The natural interpretation of the components or factors in conjunction with eigenvalues (≥ 1.5) and Scree plot determined whether a factor should be retained or not. The derived factors (pattern) were labelled on the basis of our interpretation of data and on prior literature. Each date seed variety received a score for each identified pattern.

Results

Nutritional characteristics: The level of different macro and micro nutrients (protein, fat, dietary fibers, calcium, potassium, phosphorus, magnesium, iron and zinc) are presented in Tables 1 and 2. These results were previously described elsewhere.

Antioxidant and color characteristics: Polyphenols content (total phenols, total flavonoids, catechin and epicatechin) and color are described in Table 3.

On average, the phenolic content was 3411.81 ± 717.41 mg GAE/100 g, with Khodary variety having the highest total phenolic content (4768.87 mg GAE/100g seeds), and Barhe variety having the lowest phenolic content (1864.82 mg GAE/100 g seeds).

The average of total flavonoids content was 13327.38 ± 4621.85 mg RE/100 g, with Sagay variety having the highest total flavonoid content

	Protein		Dietary Fiber		Fat	
	Mean	sd	Mean	sd	Mean	sd
All varieties						
Khalas	5.84 ^h	0.01	72.72 ^{efgh}	0.26	7.92 ^a	0.06
Barhe	5.68 ^{ef}	0.01	72.69 ^{efgh}	0.22	7.52 ^{ef}	0.05
Lulu	5.14 ^b	0.35	70.05 ^b	0.03	7.37 ^e	0.06
Shikat alkahlas	5.32 ^c	0.35	72.70 ^{efgh}	0.01	7.39 ^e	0.01
Sokkery	6.43 ⁱ	0.01	70.40 ^{bc}	0.74	6.52 ^c	0.09
Bomaan	5.38 ^c	0.01	74.00 ^{gh}	0.22	6.42 ^{bc}	0.02
Sagay	5.3 ^c	0.01	73.28 ^{efgh}	0.61	5.71 ^a	0.08
Shishi	5.70 ^{fg}	0.02	73.07 ^{efgh}	0.45	6.20 ^b	0.11
Maghool	5.55 ^{de}	0.01	73.36 ^{efgh}	0.96	6.52 ^c	0.01
Sultana	5.18 ^b	0.01	74.05 ^{gh}	0.28	6.64 ^c	0.01
Fard	5.82 ^{gh}	0.08	74.20 ^h	0.15	6.51 ^c	0.06
Maktoomi	5.83 ^h	0.01	72.07 ^{def}	0.16	7.53 ^{ef}	0.01
Naptit saif	5.70 ^{fg}	0.01	72.87 ^{efgh}	0.01	6.92 ^d	0.04
Jabri	5.42 ^{cd}	0.08	73.47 ^{fgh}	0.42	7.07 ^d	0.11
Khodary	5.36 ^c	0.04	72.52 ^{efg}	0.32	7.68 ^{fg}	0.01
Dabbas	5.13 ^b	0.03	70.89 ^{bcd}	0.21	6.93 ^d	0.02
Raziz	6.93 ^j	0.04	67.56 ^a	0.37	8.77 ^h	0.01
Shabebe	4.81 ^a	0.01	71.80 ^{cde}	0.07	7.73 ^{fg}	0.16

*Tukey test was performed to compare varieties. Different letters denote significant differences, $p < 0.05$

Table 1: Nutritional characteristics of eighteen varieties of date seeds: macronutrients content (g/100g). Means and sd are presented.*

	Ca		P		K		Mg		Fe		Zn	
	Mean	sd	Mean	sd	Mean	sd	Mean	Sd	Mean	sd	Mean	sd
All varieties												
Khalas	34.00 ^j	0.09	133.29 ^{gh}	0.02	202.19 ^{bc}	0.01	63.55 ^{abc}	0.12	3.44 ^j	0.10	1.45 ^{abc}	0.13
Barhe	30.05 ^j	0.05	122.97 ^{def}	0.01	222.19 ^{cde}	0.01	73.21 ^{defgh}	0.01	3.15 ^j	0.08	1.63 ^c	0.01
Lulu	19.89 ^{de}	0.25	134.05 ^h	2.72	204.22 ^{bcd}	1.05	68.51 ^{bode}	1.05	1.63 ^{bcd}	0.03	1.40 ^{abc}	0.14
Shikat alkahlas	20.73 ^{def}	0.54	125.82 ^{efg}	1.02	217.58 ^{cde}	1.93	86.10 ^l	1.93	1.82 ^{de}	0.04	1.13 ^{ab}	0.02
Sokkery	14.64 ^{ab}	1.09	116.87 ^{abcd}	0.87	217.92 ^{cde}	2.02	67.47 ^{abcde}	2.02	2.43 ^h	0.06	1.30 ^{abc}	0.15
Bomaan	25.06 ^h	1.07	122.39 ^{cdef}	0.31	206.82 ^{bcd}	2.91	77.01 ^{fgh}	2.91	2.14 ^g	0.05	1.53 ^{bc}	0.08
Sagay	16.85 ^{bc}	0.65	121.84 ^{cdef}	0.10	216.71 ^{cde}	4.98	65.40 ^{abcde}	4.98	2.21 ^{gh}	0.04	1.54 ^{bc}	0.19
Shishi	13.83 ^a	0.21	120.24 ^{bode}	0.12	209.49 ^{bcd}	1.23	71.57 ^{cdefg}	1.23	2.13 ^g	0.02	1.55 ^{bc}	0.17
Maghool	22.70 ^{efgh}	0.06	146.50 ^l	3.01	240.23 ^e	0.55	74.92 ^{efgh}	0.55	5.05 ^t	0.04	1.62 ^c	0.08
Sultana	18.00 ^{cd}	0.21	134.68 ^h	0.38	222.47 ^{cde}	1.56	77.61 ^{ghij}	1.56	1.40 ^{ab}	0.02	1.02 ^a	0.03
Fard	24.21 ^{gh}	0.05	115.95 ^{abcd}	0.76	238.79 ^e	1.82	72.40 ^{defgh}	1.82	2.12 ^g	0.10	1.63 ^c	0.22
Maktoomi	20.60 ^{def}	0.20	173.07 ^l	0.26	240.54 ^e	3.44	89.66 ^j	3.44	1.52 ^{abc}	0.07	1.35 ^{abc}	0.02
Naptit saif	16.13 ^{abc}	1.43	127.92 ^{efgh}	0.05	188.91 ^{ab}	3.28	76.72 ^{efgh}	3.28	1.85 ^{def}	0.17	1.31 ^{abc}	0.09
Jabri	13.41 ^{efgh}	0.47	146.77 ^l	2.32	226.28 ^{de}	0.88	81.14 ^{hij}	0.88	2.01 ^{efg}	0.10	1.20 ^{abc}	0.01
Khodary	21.81 ^{efg}	0.86	115.34 ^{abc}	1.32	213.25 ^{cd}	1.26	58.76 ^a	1.26	1.36 ^{ab}	0.06	1.42 ^{abc}	0.13
Dabbas	24.55 ^{gh}	1.10	127.91 ^{efgh}	3.13	225.98 ^{cde}	0.53	62.68 ^{ab}	0.53	1.32 ^a	0.04	1.22 ^{abc}	0.08
Raziz	20.11 ^{de}	1.24	110.09 ^a	4.65	175.02 ^a	2.83	69.54 ^{bodefg}	2.83	3.01 ^l	0.01	1.23 ^{abc}	0.14
Shabebe	24.42 ^{gh}	0.32	113.86 ^{ab}	2.38	239.40 ^e	1.49	67.63 ^{bode}	1.49	1.71 ^{cd}	0.01	1.27 ^{abc}	0.05

*Tukey test was performed to compare varieties. Different letters denote significant differences, p<0.05

Table 2: Nutritional characteristics of eighteen varieties of date seeds: micronutrients content (mg/100g). Means and sd are presented.*

	Total Phenolics (mg GAE/100g)		Total Flavonoids (mg RE/100g)		Catechin (mg /100g)		Epicatechin (mg /100g)		L		A		B	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
All varieties	3411.81	717.41	13327.38	4621.85	5.71	2.41	0.72	0.35	48.10	1.04	11.80	1.04	18.53	1.20
Khalas	2459.75 ^b	0.22	6213.33 ^a	247.40	0.76 ^a	0.02	1.16 ^j	0.11	47.23 ^c	0.09	8.43 ^a	0.19	15.13 ^a	0.19
Barhe	1864.82 ^a	12.63	7510.67 ^b	246.13	2.15 ^b	0.04	0.39 ^{bc}	0.02	48.52 ^g	0.07	10.13 ^b	0.14	16.70 ^b	0.15
Lulu	2923.11 ^e	1.27	9065.33 ^c	410.66	3.96 ^d	0.06	0.56 ^{de}	0.01	48.47 ^g	0.11	11.25 ^c	0.18	16.80 ^b	0.12
Shikat alkahlas	3129.16 ^h	0.61	10997.33 ^d	286.62	2.76 ^e	0.13	0.51 ^{cde}	0.01	48.32 ^l	0.11	11.5 ^{cd}	0.13	17.65 ^c	0.16
Sokkery	3637.45 ^t	0.45	18179.67 ^h	194.42	8.80 ^m	0.11	0.82 ^g	0.02	48.55 ^g	0.05	11.95 st	0.04	18.53 ^{de}	0.06
Bomaan	2957.95 ^g	0.69	1810.80 ^h	99.48	5.78 ^g	0.03	1.51 ^j	0.09	47.48 ^d	0.06	12.32 ^{ghij}	0.07	19.20 ^g	0.09
Sagay	3984.20 ⁿ	0.00	21673.33 ^l	105.10	7.83 ^k	0.02	0.98 ^h	0.02	47.62 ^d	0.03	12.51 ^{ij}	0.04	19.29 ^{gh}	0.05
Shishi	4087.90 ^p	0.00	15017.33 ^l	198.08	8.12 ^l	0.14	0.64 ^{et}	0.01	48.59 ^g	0.09	11.89 ^l	0.13	18.82 ^{ef}	0.13
Maghool	2940.66 ^f	0.23	9537.33 ^c	180.24	8.96 ^m	0.06	0.80 ^g	0.04	50.21 ^l	0.05	11.63 ^e	0.04	19.28 ^{gh}	0.05
Sultana	3986.63 ⁿ	1.83	14656.00 ^l	98.31	6.06 ^h	0.06	0.36 ^{bc}	0.03	46.91 ^b	0.03	12.55 ^{ij}	0.04	19.27 ^{gh}	0.08
Fard	4426.72 ^p	1.15	16113.33 ^g	387.74	6.74 ^l	0.05	0.49 ^{cde}	0.04	46.67 ^a	0.05	12.16 ^{gh}	0.09	19.28 ^{gh}	0.09
Maktoomi	3849.92 ^l	0.69	9261.33 ^c	334.72	4.4 ^e	0.01	0.47 ^{cde}	0.01	48.04 ^e	0.1	12.22 ^{gh}	0.13	18.43 ^d	0.09
Naptit saif	3422.71 ^l	2.79	12260.00 ^e	134.40	7.86 ^k	0.13	0.9 ^{gh}	0.06	48.92 ^h	0.03	11.96 ^{stg}	0.07	19.27 ^{gh}	0.13
Jabri	3860.35 ^m	0.69	16049.33 ^g	95.44	7.77 ^k	0.03	1.05 ^{ht}	0.13	47.22 ^c	0.13	12.53 ^{ij}	0.14	18.81 ^{def}	0.19
Khodary	4768.87 ^q	1.61	18157.33 ^h	194.09	7.69 ^k	0.02	1.15 ^j	0.04	46.54 ^a	0.03	12.59 ^l	0.1	18.60 ^{de}	0.17
Dabbas	2848.16 ^c	1.28	5994.67 ^a	112.09	2.66 ^c	0.05	0.19 ^a	0.01	47.58 ^d	0.13	13.06 ^k	0.23	19.81 ^l	0.20
Raziz	2866.56 ^d	0.83	18186.67 ^h	100.66	5.04 ^l	0.06	0.31 ^{ab}	0.01	48.89 ^{gh}	0.03	12.11 ^g	0.01	19.08 ^{fg}	0.03
Shabebe	3397.66i	0.46	12912.00e	128.31	6.49 ^t	0.02	0.62 ^{de}	0.02	50.10i	0.05	11.68 ^{de}	0.06	19.60 ^{hi}	0.03

*Tukey test was performed to compare varieties. Different letters denote significant differences, p<0.05

Table 3: Antioxidant and color characteristics of eighteen varieties of date seeds. Means and sd are presented.*

(21673 mg RE/100 g seed) and Dabbas variety having the lowest total flavonoid content (5995 mg RE/100 g seed).

The average contents of catechin and epicatechin were of 5.71 ± 2.41 mg/100 g and 0.72 ± 0.35 mg/100 g, respectively (Table 1). Across the different varieties, Maghool possessed the highest content of (+)-catechin (8.96 mg/100 g date seeds), while Khalas had the lowest one (0.76 mg/100 g date seeds). On the other hand, the (-)-epicatechin content was highest for Bomaan (1.51 mg/100 g date seeds) and lowest for Dabbas (0.19 mg/100 g date seeds).

The averages of L, a, and b were 48.10 ± 1.04, 11.80 ± 1.04, and

18.53 ± 1.20 respectively. Khodary had the lowest lightness score, whereas Maghool had the highest one. Khalas presented both the lowest redness-greenness and yellowness-blueness scores. Dabbas had the highest redness-greenness score and Shabebe the highest yellowness-blueness score.

Antioxidant properties

Date seeds extracts were found to possess significant total antioxidant capacity, with an average in the 18 varieties of 0.04 ± 0.02 nm (Table 4). Sultana variety had the highest antioxidant effect (0.102 nm), while Barhe (0.012) had the lowest antioxidant effect.

On average, the % superoxide inhibition was 48.40 ± 5.07 . Sagay had the highest scavenging effects (56.39% inhibition); while Khodary had the lowest scavenging effects (35.27% inhibition).

The average FRAP was 157654.25 ± 52326.08 μmol of FE/100g. The Sagay variety had the highest effect of FRAP (222569 μmol FE/100 g), while Barhe had the lowest effect (62555 μmol FE/100 g).

The average % inhibition of DPPH was 45.53 ± 7.66 . Fard possessed

the highest DPPH scavenging activity with an estimated value of 53.70%, while Barhe possessed the lowest DPPH scavenging activity with an estimated value of 29.44% (Table 5).

Correlations between nutritional, polyphenols content, color and antioxidant properties

Correlations between nutritional parameters are described in Table 6. Dietary fibers and fat content showed a significant negative

	Total Antioxidant capacity (OD, nm)		Superoxide (% inhibition)		FRAP (μmol of FE/100g)		DPPH (% inhibition)	
	Mean	s.d.	Mean	s.d.	Mean	s.d.	Mean	s.d.
All Varieties	0.04	0.02	48.40	5.07	157654.25	52326.08	45.53	7.66
Khalas	0.02 ^b	<0.01	45.70 ^{bcd}	1.56	75627.20 ^b	526.70	41.61 ^d	0.08
Barhe	0.01 ^a	<0.01	42.14 ^b	1.07	62554.93 ^a	434.48	29.44 ^a	0.03
Lulu	0.03 ^{bc}	<0.01	48.50 ^{cde}	1.49	82406.53 ^c	480.84	42.65 ^e	0.02
Shikat alkahlas	0.03 ^{bcd}	<0.01	50.64 ^{defg}	1.63	108488.93 ^e	193.71	62.90 ^m	0.77
Sokkery	0.03 ^{bc}	<0.01	53.05 ^{efgh}	1.63	183802.80 ⁱ	388.09	44.43 ^g	0.02
Bomaan	0.05 ^{gh}	<0.01	44.57 ^{bc}	1.10	137681.87 ^g	348.50	42.85 ^e	0.00
Sagay	0.05 ^{fgh}	<0.01	56.39 ^h	2.58	222569.20 ⁿ	219.07	52.73 ^k	0.01
Shishi	0.04 ^{fg}	<0.01	48.43 ^{cde}	1.59	171449.07 ⁱ	762.89	47.18 ^j	0.02
Maghool	0.03 ^{bc}	<0.01	47.73 ^{cd}	1.20	159825.07 ^h	203.22	35.57 ^b	0.02
Sultana	0.10 ⁱ	<0.01	47.77 ^{cd}	1.51	210160.27 ^m	621.99	48.81 ^j	0.01
Fard	0.05 ^h	<0.01	55.32 ^{gh}	1.95	198639.20 ^j	15.24	53.70 ^j	0.03
Maktoomi	0.04 ^{fg}	<0.01	44.47 ^{bc}	1.56	102144.13 ^d	170.67	43.03 ^e	0.03
Naptit Saif	0.04 ^{def}	<0.01	48.28 ^{cde}	2.13	199638.13 ⁱ	576.56	42.65 ^e	0.01
Jabri	0.05 ^h	<0.01	53.59 ^{fgh}	1.25	221595.73 ⁿ	724.60	43.68 ^f	0.02
Khodary	0.05 ^{gh}	<0.01	35.27 ^a	1.28	221467.07 ⁿ	509.95	53.08 ^k	0.04
Dabbas	0.03 ^{cde}	<0.01	50.67 ^{defg}	2.08	159552.27 ^h	675.46	53.16 ^{kl}	0.02
Raziz	0.07 ⁱ	0.01	48.98 ^{cdef}	1.19	188095.60 ^k	538.92	45.32 ^h	0.00
Shabebe	0.04 ^{ef}	<0.01	49.72 ^{def}	1.61	132078.53 ^f	108.76	36.71 ^c	0.00

*Tukey test was performed to compare varieties. Different letters denote significant differences, $p < 0.05$

Table 4: Antioxidant capacities of eighteen varieties of date seeds. Means and sd are presented*.

	Protein	Fiber	Fat	Ca	P	K	Mg	Fe	Zn
Protein	1	-0.03	0.07	-0.12	-0.10	-0.22	0.09	0.60*	0.30
Fiber	-0.03	1	-0.60**	-0.04	0.26	0.21	0.43	0.15	0.28
Fat	0.07	-0.60**	1	0.35	-0.04	-0.10	-0.12	-0.14	-0.34
Ca	-0.12	-0.04	0.35	1	-0.11	0.12	-0.22	0.13	0.32
P	-0.10	0.26	-0.04	-0.11	1	0.28	0.53*	-0.12	-0.17
K	-0.22	0.21	-0.10	0.12	0.28	1	0.28	-0.21	-0.10
Mg	0.09	0.43	-0.12	-0.22	0.53*	0.28	1	-0.3	-0.19
Fe	0.60*	0.15	-0.14	0.13	-0.12	-0.21	-0.3	1	0.53*
Zn	0.30	0.28	-0.34	0.32	-0.17	-0.10	-0.19	0.53*	1

*Spearman's correlation coefficients were calculated. Statistical significance set at $p < 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 5: Correlations between nutritional parameters among 18 varieties of date seeds.*

	Total Phenolic	Total flavonoid	Catechin	Epicatechin	L	a	B	TAC	Superoxide	FRAP	DPPH
Total Phenolics	1	0.59*	0.61**	0.21	-0.39	0.52*	0.491*	0.63**	0.20	0.70**	0.53*
Total flavonoids	0.59*	1	0.58*	0.30	-0.12	0.42	0.581*	0.73**	0.34	0.73**	0.38
Catechin	0.61**	0.58*	1	0.38	0.27	0.22	0.659**	0.33	0.26	0.67**	0.04
Epicatechin	0.21	0.30	0.38	1	-0.16	-0.03	0.174	0.03	-0.12	0.21	-0.22
L	-0.39	-0.12	0.27	-0.16	1	-0.54*	0.20	-0.37	0.01	-0.28	-0.52*
A	0.52*	0.42	0.22	-0.03	-0.54*	1	0.207	0.70**	0.17	0.70**	0.57*
B	0.49*	0.58*	0.66**	0.17	0.20	0.21	1	0.61*	0.26	0.56*	-0.01
TAC	0.63**	0.73**	0.33	0.03	-0.37	0.70**	0.61*	1	0.17	0.75**	0.43
Superoxide	0.20	0.34	0.26	-0.12	0.01	0.17	0.259	0.17	1	0.41	0.46
FRAP	0.70**	0.73**	0.67**	0.21	-0.28	0.70**	0.562*	0.75**	0.41	1	0.49*
DPPH	0.53*	0.38	0.04	-0.22	-0.52*	0.57*	-0.011	0.43	0.46	0.49*	1

*Spearman's correlation coefficients were calculated. Statistical significance set at $p < 0.05$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

Table 6: Correlations between phenolics, flavonoids, catechin, epicatechin, antioxidant capacity and color among 18 varieties of date seeds.*

correlation. By contrast, protein and iron levels, phosphorus and magnesium, and finally, iron and zinc were positively correlated.

Spearman correlation coefficients between polyphenols content, color and antioxidant properties are presented in Table 7. Total phenolics were positively and significantly correlated with the total antioxidant capacity, FRAP, and DPPH. Total flavonoids were positively and significantly related to the total antioxidant capacity and FRAP. Catechin was positively and significantly related to FRAP. In contrast, epicatechin was not correlated to any parameter. A lower lightness was associated with higher redness-greenness and higher DPPH. Results showed that the higher the redness-greenness color, resulted in higher phenolics, total antioxidant capacity, FRAP and DPPH. The higher the yellowness-blueness color, the higher the content of phenolics, flavonoids, catechin, total antioxidant capacity, and FRAP.

A higher yellowness-blueness color was related to a lower level of dietary fiber. Also, if the catechin content was high, the fat content would be low. The results also showed that the higher the calcium content, the higher the total phenolics and catechin contents, but lower FRAP content. Phosphorus and total flavonoids were inversely correlated. Iron was only inversely related to redness-greenness color. Magnesium and zinc were not related to any of the antioxidant parameters.

Factorial analysis: identification of nutrition, antioxidant and color based patterns for date seeds

Factorial analysis identified two main factors with eigenvalues of 5.84 and 2.83 for factor 1 and factor 2, respectively. The two factors were explaining almost 45% of total variance. The matrix of components scores is in Table 8.

The first factor was determined by total phenolics, total flavonoids, catechin, a, b, TAC, FRAP, DPPH in a positive manner and with fat, calcium in a negative manner.

The second factor was determined by epicatechin, dietary fibers, iron, and zinc in a positive way and by fat in a negative way.

The correlations of the eighteen date seeds varieties with these two factors enabled the identifications of the specificities of the different varieties and to highlight 4 different patterns of date seeds varieties. The results are presented in Figure 1. Some varieties, including Sagay, Fard, Sultana, Jabri and Khodary were mainly characterized by high levels of phenolics, flavonoids, catechin, higher redness-greenness color, higher yellowness-blueness color, higher TAC, FRAP, and DPPH, but low fat and calcium contents. However, for the Khalas and Barhe varieties it was the opposite. The Maghool variety was stood out because it was strongly associated with high epicatechin, dietary fibers, iron, and zinc contents but with low fat level. By contrast, the Shikat alkalhas, Dabbas

and Raziz varieties were characterized by low epicatechin, dietary fibers, iron, and zinc contents, but by high fat level. These results were confirmed by conducting a cluster analysis used to classify the different varieties according to the same nutritional and antioxidant parameters as those which were considered in the previous factorial analysis.

Discussion

This work is the first of its kind to describe the nutritional and antioxidant properties, color of 18 different varieties of date seeds, and to determine the specificities of each variety of date seeds. Particularly high levels of dietary fibers, phenolics, and flavonoids were obtained as well as high antioxidant activities, as evidenced by both strong reducing power and radical-scavenging capacity. Interestingly, combinations of nutritional and antioxidant specificities were identified in date seeds varieties. Some varieties were better characterized by their fat and calcium contents, antioxidant components, antioxidant effects, yellowness-blueness and redness-greenness color; whereas the others were rather characterized by epicatechin, dietary fibers, iron and zinc contents.

The nutritional characteristics of the same date seeds varieties have already been discussed in details somewhere else and emphasized the particularly high content in dietary fibers. This is interesting in terms of prevention of chronic diseases like cardiovascular diseases or diabetes [3].

The polyphenolic compounds content and antioxidant effects of the date seeds were additionally studied in this work. The findings which were obtained are in agreement with the results reported by other studies previously conducted on date seeds [25-27]. Remarkably, the content of polyphenols and the antioxidant activity were much higher, up to 10-fold, in date seeds compared to date fruits, but also compared to other popular dietary antioxidant-rich food products like tea extract or some other by-products like grape seeds [17,25,28].

The strength of this work is to have discriminated the date seeds varieties on the basis of their nutritional, antioxidant, and color properties, and to have determined which nutritional and antioxidant properties better characterize each variety. In this way, it was possible to highlight group or patterns of varieties on the basis of their nutritional, antioxidant and color properties. These parameters were interrelated in date seeds but in a different manner according to the variety. These differences can be explained by different factors such as; genetic, environmental and agricultural factors including time of harvest, post-harvest treatments, fertilizers, quality of irrigation water, and variable soil mineral availability. In regards to polyphenols, the presence of water-soluble compounds with different free radical-scavenging effects, such as phenolics and flavonoids, could help explain the differences

	Total Phenolic	Total flavonoid	Catechin	Epicatechin	L	a	B	TAC	Superoxide	FRAP	DPPH
Protein	-0.01	0.21	0.16	0.09	0.13	-0.23	-0.08	-0.03	-0.12	-0.01	-0.13
Fiber	0.44	0.18	0.31	0.32	-0.40	0.22	0.48*	0.37	0.04	0.39	0.12
Fat	-0.42	-0.33	-0.58*	-0.20	0.05	-0.25	-0.41	-0.19	-0.37	-0.40	-0.25
Ca	-0.55*	-0.46	-0.63*	-0.08	-0.14	-0.24	-0.23	-0.37	-0.25	-0.61*	-0.25
P	-0.16	-0.58*	-0.14	0.01	-0.11	-0.03	-0.29	-0.25	-0.05	-0.19	-0.28
K	0.22	-0.20	0.13	-0.29	-0.06	0.19	0.18	-0.05	0.17	-0.02	-0.01
Mg	0.10	-0.10	0.01	-0.15	0.06	-0.04	0.14	0.17	-0.13	-0.07	-0.09
Fe	-0.36	0.15	0.17	0.28	0.37	-0.55*	0.13	-0.26	-0.01	-0.14	-0.45
Zn	0.01	0.01	0.17	0.27	0.06	-0.32	0.15	-0.22	-0.26	-0.17	-0.30

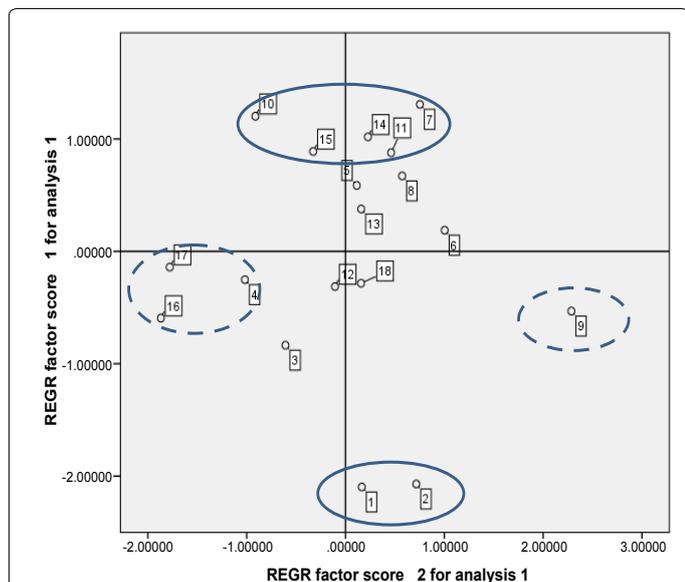
*Spearman's correlation coefficients were calculated: Statistical significance set at p<0.05, *p<0.05, **p<0.01, ***p<0.001

Table 7: Correlations between color, nutritional and antioxidant parameters among 18 varieties of date seeds.

Component	1	2
Eigenvalue	5.84	2.83
% of variance explained	29.19	14.14
Total Phenolics	0.86	0.04
Total Flavonoids	0.81	0.03
Catechin	0.77	0.40
Epicatechin	0.18	0.51
L	-0.31	0.20
a	0.79	-0.20
B	0.52	0.41
TAC	0.69	-0.30
Superoxide	0.34	-0.02
FRAP	0.89	-0.06
DPPH	0.51	-0.44
Protein	-0.05	-0.21
Fiber	0.27	0.69
Fat	-0.52	-0.55
Ca	-0.77	0.09
P	-0.15	0.26
K	0.07	0.45
Mg	0.08	0.17
Fe	-0.42	0.50
Zn	-0.22	0.70

Factorial analysis with Principal Component Analysis and orthogonal rotation was performed. Absolute Eigenvalue ≥ 0.5 is bolded

Table 8: Matrix for the two extracted components.*



*Factorial analysis with Principal Component Analysis and orthogonal rotation was performed.

Variety	Number	Variety	Number
Khalas	1	Sultana	10
Barhe	2	Fard	11
Lulu	3	Maktoomi	12
Shikat alkahlas	4	Naptit Saif	13
Sokkery	5	Jabri	14
Bomaan	6	Khodary	15
Sagay	7	Dabbas	16
Shishi	8	Raziz	17
Maghool	9	Shabebe	18

Figure 1: Date seeds patterns based on nutritional, antioxidant and color characteristics.*

obtained, in particular for superoxide anion (O_2^-) scavenging power, FRAP and DPPH. Indeed, these compounds, especially catechin and

epicatechin, are the first to be oxidized contributing to a lower radical-scavenging activity. This process has already been described by Chaira [29].

On a side note, it appeared as though some varieties were better characterized by their fat and calcium contents, antioxidant components, antioxidant effects, and yellowness-blueness and redness-greenness color. Not surprisingly, phenolics and flavonoids were strongly and positively related to the antioxidant capacities, both radical scavenging and reducing powers. Interestingly, catechin but not epicatechin, was positively correlated with reducing the radical scavenging powers. This may be related to the relatively low concentration of epicatechin in date seeds compared to other plant products.

The varieties presenting the highest antioxidant capacity are also those with the lowest amount of fat, which is particularly important in the purpose of the use of date seeds as a functional ingredient. The date seeds that contained the highest levels of phenolics and flavonoids enabled the maximum antioxidant effect of polyphenols by minimizing fat intake and hence contribute to prevention of diseases like cardiovascular diseases, diabetes and cancer. This is said to be the cause of the potent antioxidant and anticancer activities of phenolics and flavonoids [30].

Our results suggest that the color of the date seeds can be used as an indicator of the nutritional and antioxidant properties. Indeed, colorfulness seems to indicate higher content of phenolics, flavonoids and catechin, higher TAC, FRAP and DPPH, but a lower fat and calcium. Color parameters were only recently considered in the study of antioxidant properties of food, and our results are in accordance with other studies related to cereal products such as rice or fruits such as pomegranate [31,32]. Coloration has been related to anthocyanins, which were not measured in our work. Nonetheless, a higher level can be expected in darker varieties compared to lighter ones.

On the other hand, other varieties were better characterized by their epicatechin, dietary fibers, fat, iron, and zinc levels; with the highest contents in epicatechin, dietary fibers, zinc, and iron being observed in the same varieties as those with the lowest fat level. Metal minerals have been demonstrated to play a significant beneficial role in oxidative stress and inflammation in many chronic diseases [33]. Knowing this and considering the known health benefits of fibers; the combination of these nutritional properties in the same varieties, make these latter good candidate for prevention and management of diseases related to oxidative stress. Further, this information can be used in the prevention of diseases more related to the effect of dietary fibers like diabetes and gastrointestinal cancers.

Conclusion

The results of this study suggest that date seeds could be a potential promising candidate for future pharmaceutical application or functional food in order to prevent and treat illnesses and improve overall health, according to their nutritional and antioxidant characteristics. But, interestingly, it indicated also that this potential varied among the date seeds varieties, with different nutritional and antioxidant profiles. This is of a particularly great importance, when considering a specific health purpose, for the selection of date seeds varieties, single or combination of varieties, in the development of functional food. Further research is needed to start developing functional products based on date seeds and to test the effect of single varieties and combinations of varieties.

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