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Comparison of Antioxidant Activity, Anthocyanins, Carotenoids, and Phenolics of Three Native Fresh and Sun-Dried Date (*Phoenix dactylifera* L.) Varieties Grown in Oman

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Fresh and sun-dried dates of three native varieties from Oman, namely, Fard, Khasab, and Khalas, were examined for their antioxidant activity and total contents of anthocyanins, carotenoids, and phenolics, as well as free and bound phenolic acids. All results are expressed as mean value ± standard deviation ($n = 3$) on a fresh weight basis. Fresh date varieties were found to be a good source of antioxidants (11687−20604 μmol of Trolox equiv/g), total contents of anthocyanins (0.24−1.52 mg of cyanidin 3-glucoside equiv/100 g), carotenoids (1.31−3.03 mg/100 g), phenolics (134−280 mg of ferulic acid equiv/100 g), free phenolic acids (2.61−12.27 mg/100 g), and bound phenolic acids (6.84−30.25 mg/100 g). A significant ($p < 0.05$) amount of antioxidants and carotenoids was lost after sun-drying of dates, whereas the total content of phenolics and free and bound phenolic acids increased significantly ($p < 0.05$). Anthocyanins were detected only in fresh dates. Date varieties had different levels and patterns of phenolic acids. Four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) and nine bound phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, ferulic acid, and *o*-coumaric acid) were tentatively identified. Of the date varieties studied, Khalas, which is considered to be premium quality, had higher antioxidant activity, total carotenoids, and bound phenolic acids than other varieties. These results suggest that all date varieties serve as a good source of natural antioxidants and could potentially be considered as a functional food or functional food ingredient, although some of their antioxidant constituents are lost during sun-drying.

KEYWORDS: Fresh and sun-dried dates; antioxidant; anthocyanins; carotenoids; phenolics; phenolic acids

INTRODUCTION

Dates are highly popular worldwide, particularly in the Middle East and North Africa. Oman produced 231 000 metric tons of dates in 2004, contributing ∼3.4% to the total global production. The per capita daily consumption of dates in Oman is estimated at 55−164 g (*1*), and dates are considered to be a vital component of the daily diet. In Oman, dates are consumed either fresh (30−40%) or sun-dried (60−70%). Sun-dried dates are consumed throughout the year, but their use reaches a peak during Ramadan for breaking the fast before eating. It is, therefore, of great interest to assess and compare the antioxidant and functional characteristics of both fresh and sun-dried date varieties.

High fruit and vegetable consumption is associated with a reduced risk of several chronic diseases such as cancer, cardiovascular disease, coronary heart disease, and atherosclerosis, as well as neurodegenerative disease and inflammation (*2*−*6*). The compounds thought to be responsible for the protective effects of a fruit- and vegetable-rich diet include carotenoids and antioxidant vitamins. However, there is growing evidence that other phytochemicals (non-nutritive components) contribute to varying degrees to the antioxidant activity of individual fruits and vegetables. In this regard, attention has been focused on the significance of phenolics such as phenolic acids, flavonoids, and in particular anthocyanins (*4*, *6*−*10*). Antioxidants can be classified into two groups according to their solubility; hydrophilic antioxidants (water-soluble), such as the majority of phenolic compounds and ascorbic acid, and lipophilic antioxidants (fat-soluble) such as carotenoids and vitamin E (*11*).

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Sun-drying is a traditional way of preserving dates (*12*). Although most of the dates produced are sun-dried (60−70%), there is no study covering the direct effect of sun-drying on the nutritional and functional properties of dates. However, the effect of drying on fruit antioxidants is not fully understood, although a few papers have been published on these subjects (*13*−*15*). Therefore, detailed information about the health-promoting components of dates could lead to a better understanding and an increased consumption, including their use as functional foods and ingredients in nutraceuticals, pharmaceuticals, and medicine. The objectives of this research were to compare the existing differences in antioxidant components of three native fresh and sun-dried date varieties grown in Oman and to assess the effect of sun-drying on their activity.

MATERIALS AND METHODS

Date Samples. The fresh and sun-dried (commercial way of drying) date varieties, namely, Fard (red as fresh and dark red as sun-dried), Khasab (red as fresh and dark red as sun-dried), and Khalas (yellow as fresh and dark gold as sun-dried) were procured from a local farm in Fanjh, Oman, at the beginning of the 2003 harvest season. The quality standards for these dates have been well separated in Oman in accordance with their usage, sensory characteristics, and prices. Khalas is regarded as being of premium quality due to its sensory quality and high price. The sensory quality and price of Khasab are lower than those of Khalas. Thus, Khasab is perceived as being of moderate quality. Fard is known as industrial quality and mainly used for processing purposes. Fresh (1 day after harvesting) and sun-dried (at 30−50 °C for 7−10 days) date varieties used in this study were obtained from the same batches. They were dispatched (packed into an insulated polystyrene box with cooling gel) by TNT World Wide Express to the Food Research Center, University of Lincoln (Lincoln, U.K.). Mature fruits of uniform size, free of physical damage and injury from insects and fungal infection, were selected and used for all experiments. Upon arrival at the laboratory, the samples (100−150 g portions) were packed in polyethylene bags, sealed, and stored at −30 °C until analyzed.

Chemicals. All chemicals were obtained from Sigma-Aldrich Co. Ltd. (Dorset, U.K.), unless otherwise specified.

Extraction Methods. There are no satisfactory solvent extraction methods suitable for the isolation of all classes of food antioxidants and phenolics or even for a specific class of these components. This is due to the chemical nature of food antioxidants and phenolics, which vary from being simple to being very highly polymerized (*6*). Therefore, the extraction of antioxidant compounds and total phenolics from one of the sun-dried date varieties (Fard, as a representative variety) was carried using seven different solvents, which had been used in previous studies (*16*−*20*). The Fard sample (1 g) was extracted using 40 mL of H_2O, phosphate buffer (75 mM, pH 7.4), methanol (containing 0.1% formic acid)/H_2O (88:12, v/v), methanol/HCl (99.9:0.1, v/v), acetone/H_2O (70:30, v/v), acetone (containing 7% cyclodextrin)/H_2O (50:50, v/v), or methanol/H_2O (50:50, v/v).

Measurement of Oxygen Radical Absorbance Capacity (ORAC). An improved ORAC method of Ou et al. (*16*), using fluorescein (FL) as the fluorescent probe, was used with slight modifications. The $ORAC_{FL}$ assay measures the ability of antioxidative compounds in test materials to inhibit the decline in fluorescence induced by peroxyl radical 2,2′-azobis(2-amidinopropane) dihydrochloride (AAPH). Briefly, a 150 μL sample extract was introduced into a 3 mL fluorescence cell, followed by 150 μL of 0.12 μM disodium FL solution, and 2.55 mL of 75 mM phosphate buffer (pH 7.4). Phosphate buffer was used as a blank, and Trolox (a water-soluble α-tocopherol analogue) at 2.5, 5, and 10 μM was used as standard. The cell was incubated at 37 °C for 15 min in a water bath. The initial fluorescence (f_0) was measured at the excitation wavelength of 493 nm and the emission wavelength of 515 nm using an RF-540 Shimadzu spectrofluorophotometer (Shimadzu, Kyoto, Japan). After f_0 had been recorded, 150 μL of 320 mM AAPH reagent, as a free radical generator, was added into a cell and mixed well using a glass rod. Fluorescence was measured and recorded every 5 min (f_5, f_{10}, f_{15}, ..., f_{60}) until the fluorescence of the last reading declined by >95% from the first reading (~60 min). The area under the curve (AUC) and relative $ORAC_{FL}$ values were calculated according to the method of Wang et al. (*64*), as shown below. $ORAC_{FL}$ values are expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight.

$$\mathrm{AUC} = [0.5 + (f_5/f_0) + (f_{10}/f_0) + ... + (f_{60}/f_0)] \times 5$$

$$\mathrm{ORAC_{FL}\ value} = \frac{\mathrm{AUC_{sample}} - \mathrm{AUC_{blank}}}{\mathrm{AUC_{Trolox}} - \mathrm{AUC_{blank}}} \times \text{Trolox molarity} \times \text{sample dilution}$$

Measurement of Total Phenolics. Total phenolics were determined colorimetrically using Folin−Ciocalteau reagent as described by Velioglu et al. (*21*), with slight modifications. Two hundred milligrams of sample was extracted for 2 h with 2 mL of 50% methanol at room temperature on an orbital shaker set at 200 rpm. The mixture was centrifuged at 1000*g* for 15 min, and the supernatant was decanted into 4 mL vials. The pellets were extracted under identical conditions. Supernatants were combined and used for total phenolic assay. The extract (200 μL) was mixed with 1.5 mL of Folin−Ciocalteau reagent (previously diluted 10-fold with distilled water) and allowed to stand at 22 °C for 5 min. A 1.5 mL sodium bicarbonate solution (60 g/L) was added to the mixture. After 90 min at 22 °C, absorbance was measured at 725 nm using a UV-1601 Shimadzu spectrophotometer (Shimadzu). Total phenolics were quantified by calibration curve obtained from measuring the absorbance of a known concentration of ferulic acid standard (1−100 mg/100 mL of 50% methanol). The concentrations are expressed as milligrams of ferulic acid equivalents (FAE) per 100 g of fresh weight.

Measurement of Total Anthocyanins. Total anthocyanins were determined according to the pH-differential method of Guisti and Wrolstad (*22*). All manipulations were carried out under a yellow fluorescent light (Thorn Lighting Limited, Herts, U.K.), to avoid light-induced changes. Approximately 1 g of date sample was homogenized in 80 mL of distilled water for 1 min and then sonicated for 15 min until the sample was completely dissolved. After that, the extract was centrifuged at 1500*g* for 10 min to remove particulates. Clear extract (1 mL) was placed into a 25 mL volumetric flask, made up to a final volume with pH 1.0 buffer (1.49 g of KCl/100 mL of water and 0.2 N HCl, with a ratio of 25:67), and mixed. Another 1 mL of extract was also placed into a 25 mL volumetric flask, made up to a final volume with pH 4.5 buffer (1.64 g of sodium acetate/100 mL of water), and mixed. Absorbance was measured in a UV-1601 Shimadzu spectrophotometer (Shimadzu) at 510 nm and at 700 nm. Absorbance was calculated as $\mathrm{Ab} = (A_{510nm} - A_{700nm})\mathrm{pH}_{1.0} - (A_{510nm} - A_{700nm})\mathrm{pH}_{4.5}$ with a molar extinction coefficient for cyanidin 3-glucoside of 26900. Results were calculated using the following equation and expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight.

$$\text{total anthocyanins (mg/100 g)} = \frac{\mathrm{Ab}}{eL} \times \mathrm{MW} \times \mathrm{D} \times \frac{V}{G} \times 100$$

where Ab is absorbance, *e* is cyanidin 3-glucoside molar absorbance (26900), *L* is cell path length (1 cm), MW is the molecular weight of anthocyanins (449.2), D is a dilution factor, *V* is the final volume (mL), and *G* is the sample weight (mg).

Measurement of Total Carotenoids. Total carotenoids were extracted according to the method of Talcott and Howard (*23*), with slight modifications. Two grams of sample was extracted using 25 mL of acetone/ethanol (1:1, v/v) with 200 mg/L butylated hydroxytoluene (BHT) added. All manipulations were carried out under a yellow fluorescent light (Thorn), to avoid light-induced changes. After extraction, sample was centrifuged at 1500*g* for 15 min at 4−5 °C. The supernatant was collected, and the remaining residue was re-extracted using the same method until the residue was colorless. Finally, the combined supernatants were brought to 100 mL with the extraction solvent, and the absorbance at 470 nm was measured using a UV-1601 Shimadzu spectrophotometer (Shimadzu). Total carotenoids were calculated according to the method of Gross (*24*), using the following

Table 1. Comparison of Extraction Solvents for the Contents of Antioxidant Activity and Phenolics in Sun-Dried Date Variety Fard[a]

extraction solvent	antioxidant activity[b] (μmol of TE/g)	total phenolics[c] (mg of FAE/100 g)
H_2O	9177 ± 798d	276 ± 5d
phosphate buffer (75 mM, pH 7.4)	9986 ± 765e	292 ± 9d
methanol (containing 0.1% formic acid)/H_2O (88:12, v/v)	8552 ± 650f	248 ± 12e
methanol/HCl (99.9:0.1, v/v)	2005 ± 191g	308 ± 9f
acetone/H_2O (70:30, v/v)	9406 ± 115d	280 ± 9d
acetone (containing 7% cyclodextrin)/H_2O (50:50, v/v)	7312 ± 484h	314 ± 8f
methanol/H_2O (50:50, v/v)	5840 ± 343i	343 ± 7g

[a] Data are expressed as mean ± SD ($n = 3$) on a fresh weight basis. Means ± SD followed by the same letter, within a column, are not significantly different ($p > 0.05$). [b] Antioxidant activity, expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight. [c] Total phenolics, expressed as milligrams of ferulic acid equivalents (FAE) per 100 g of fresh weight.

equation, and expressed as milligrams per 100 g of fresh weight.

$$\text{total carotenoids (mg/g)} = \frac{\text{Ab} \times V \times 10^6}{A^{1\%} \times 100G}$$

Ab is the absorbance at 470 nm, V is the total volume of extract, $A^{1\%}$ is the extinction coefficient for a 1% mixture of carotenoids at 2500, and G is sample weight (g).

Extraction, Hydrolysis, Identification, and Quantification of Phenolic Acids. Phenolic acids in date varieties were determined according to the high-performance liquid chromatographic (HPLC) method of Mattila and Kumpulainen (*25*) and Alasalvar et al. (*26*). Extraction, hydrolysis (free, alkaline, and acid), identification, and quantification of phenolic acids together with HPLC column, pump, diode array detector, and autosampler used were the same as those described in a previous study (*26*). Tentatively identified phenolic acids were quantified on the basis of their peak areas and comparison with a calibration curve obtained with the corresponding standards (standard concentrations ranging from 1 to 1000 μg/mL of methanol). The results for free, alkaline, and acid hydrolysates were calculated to represent total phenolic acids. Free and bound phenolic acids are expressed as milligrams per 100 g of fresh weight.

Statistical Analysis. Results are expressed as mean value ± standard deviation (SD) ($n = 3$) on a fresh weight basis. Statistical significance (*t* test: two-sample equal variance, using two-tailed distribution) was determined using Microsoft Excel data analysis. Differences at $p < 0.05$ were considered to be significant.

RESULTS AND DISCUSSION

Extraction Methods. Table 1 compares the effect of extraction methods on antioxidant activity and content of total phenolics in sun-dried dates (Fard), using seven different solvents. Only one variety was used as a representative of dates to evaluate the solvent extraction process. Significant ($p < 0.05$) differences existed among different solvents used, with some exceptions. Extraction into phosphate buffer (75 mM, pH 7.4) gave the highest antioxidant activity (9986 μmol of TE/g), whereas methanol/HCl (99.9:0.1, v/v) afforded the lowest (2005 μmol of TE/g) antioxidant activity among the solvents used. These results suggest that most of the antioxidants in dates are water-soluble (hydrophilic). In contrast to antioxidant activity, methanol/H_2O (50:50, v/v) yielded the highest recovery of total phenolics (343 mg of FAE/100 g), whereas that in methanol [containing 0.1% formic acid/H_2O (88:12, v/v)] was the lowest (248 mg of FAE/100 g). This was due to the solubility differences of phenolic acids in methanol, water, or their mixtures (*25*, *27*, *28*). Thus, phosphate buffer for antioxidant activity and methanol/H_2O (50:50, v/v) for total phenolics was selected to extract the remaining date varieties.

$ORAC_{FL}$. Significant differences ($p < 0.05$) in $ORAC_{FL}$ values were observed among fresh and sun-dried date varieties (**Table 2**). The antioxidant activity among fresh dates was in the range of 11687−20604 μmol of TE/g, being lowest in Khasab and highest in Khalas. The higher $ORAC_{FL}$ values obtained in this study compared to that of the previously described ORAC method of Cao et al. (*29*) was due to the use of fluorescein as the fluorescent probe, which was developed by Ou et al. (*16*). **Figure 1** shows the fluorescence decay induced by AAPH during the assay time for blank, standards, and sun-dried dates of the Fard variety.

Ou et al. (*16*) measured the antioxidant activity of bilberry and elderberry using the $ORAC_{FL}$ assay, and the values were 2646 and 2221 μmol of TE/g of fresh weight, respectively. Recently, Alasalvar et al. (*26*) reported that the $ORAC_{FL}$ values of two native fresh cherry laurel varieties and pekmez (concentrated juice of cherry laurel produced by boiling/heating) ranged from 3363 to 19981 μmol of TE/g of fresh weight. Thus, in comparison with these fruits and pekmez, date varieties were found to be a rich source of antioxidants. This finding is also supported by other studies published earlier on date antioxidants (*30*, *31*). Although Vayalil (*30*) and Guo et al. (*31*) used different methods and extraction solvents, which make any quantitative comparison difficult, Vayalil (*30*) stated that potent antioxidant and antimutagenic activities of dates implicate the presence of free radical scavengers. In addition, Guo et al. (*31*) reported that dates had the second highest antioxidant activity among 28 fruits commonly consumed in China.

Sun-drying caused a significant loss (ranging from 29.7 to 42.5%) of antioxidant activity in date varieties (**Figure 2**). This loss could be due to the decomposition of natural antioxidants in dates after drying. The reduction in antioxidant activity after drying has also been reported for other fruits. Larrauri et al. (*32*) reported decreases of 28 and 50% in the antioxidant activity of red grape pomace peel after drying at temperatures of 100 and 140 °C, respectively. The antioxidant activity of blueberry decreased after drying (52%) and canning (65%) (*33*). The difference between fresh and sun-dried date antioxidants observed in this study was of a similar magnitude. In contrast, there are a few studies in which either an increase in antioxidant activity or no change after drying was observed. Piga et al. (*14*) reported an increase in antioxidant activity in plums and prunes after drying at 85 °C for 40 h. They explained that this increase was due to the formation of Maillard reaction products during drying process. Thus, chemical and biochemical changes that affect the antioxidant activity may occur during drying.

Total Anthocyanins. Anthocyanins, which were detected only in fresh dates, showed significant differences ($p < 0.05$) among the varieties examined. The highest content of anthocyanins was present in Khasab (1.52 mg/ 100 g), followed by Fard (0.92 mg/100 g) and Khalas (0.24 mg/100 g), expressed as cyanidin 3-glucoside equivalents. These differences are related to the color of these varieties (both Khasab and Fard varieties are red, whereas Khalas variety is yellow due to the presence of higher content of carotenoids).

The anthocyanin contents of a number of fruits and different cultivars of the same fruits have been reported by several researchers (*33*−*36*). Total anthocyanin contents of several fruits and their cultivars reported by these researchers ranged widely from 0 to 515 mg of cyanidin 3-glucoside equiv/100 g of fresh weight, being absent in white grape cultivars (*34*) and highest in 30 genotypes of *Vaccinium* L. (blueberries), respectively (*36*).

Table 2. Contents of Antioxidant Activity ($ORAC_{FL}$), Phenolics, Anthocyanins, and Carotenoids in Fresh and Sun-Dried Date Varieties[a]

	Fard		Khasab		Khalas	
	fresh	sun-dried	fresh	sun-dried	fresh	sun-dried
$ORAC_{FL}$[b] (μmol of TE/g)	17377 ± 836f	9986 ± 765g	11687 ± 309h	8212 ± 515i	20604 ± 524j	12543 ± 339k
total anthocyanins[c] (mg/100 g)	0.92 ± 0.09f	nd	1.52 ± 0.13g	nd	0.24 ± 0.05h	nd
total carotenoids[d] (mg/100 g)	1.39 ± 0.08f	1.19 ± 0.11g	1.31 ± 0.03h	0.92 ± 0.11i	3.03 ± 0.05j	2.91 ± 0.05j
total phenolics[e] (mg of FAE/100 g)	280 ± 6f	343 ± 7g	167 ± 5h	217 ± 2i	134 ± 6j	339 ± 3g

[a] Data are expressed as mean ± SD ($n = 3$) on a fresh weight basis. The moisture contents of Fard, Khasab, and Khalas were 48.8, 43.6, and 33.4% (fresh) and 18.5, 16.5, and 12.6% (sun-dried), respectively. Means ± SD followed by the same letter, within a row (Fard, Khasab, and Khalas), are not significantly different ($p > 0.05$). nd, not detected. [b] Antioxidant activity ($ORAC_{FL}$), expressed as micromoles of Trolox equivalents (TE) per gram of fresh weight. [c] Total anthocyanins, expressed as milligrams of cyanidin 3-glucoside equivalents per 100 g of fresh weight. [d] Total carotenoids, expressed as milligrams per 100 g of fresh weight. [e] Total phenolics, expressed as milligrams of ferulic acid equivalents (FAE) per 100 g of fresh weight.

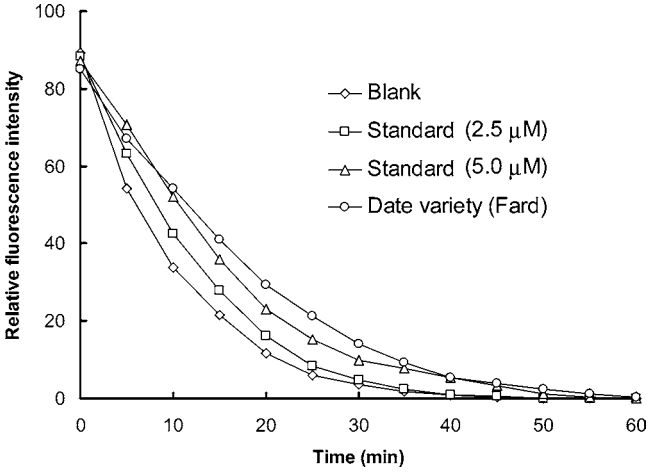

Figure 1. Fluorescence decay curve induced by AAPH.

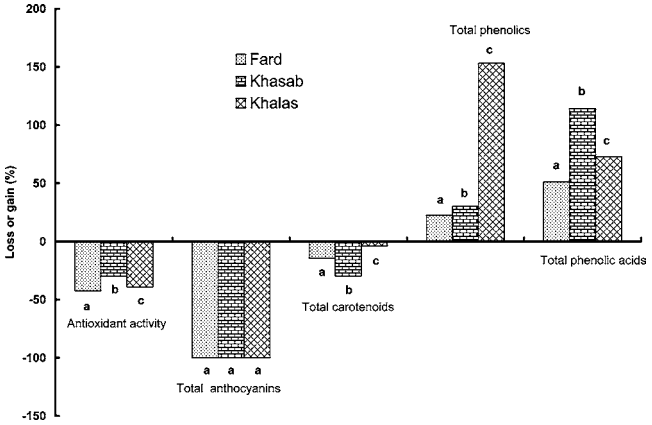

Figure 2. Effect of sun-drying on date antioxidants.

Within these ranges, date varieties may be considered to be poor sources of anthocyanins. However, blue, red, violet, and purple colors of most edible plant species and their fruits (berries, grapes, cherries, cherry laurels, plums, etc.) are due to anthocyanins (*6*, *7*, *26*, *37*, *38*).

No anthocyanins were detected in sun-dried dates, possibly due to their destruction upon drying (**Table 2** and **Figure 2**). In a previous study (*26*), we found that a significant amount of anthocyanins was lost (92.5%) during heat processing in the production of pekmez. Raynal (*39*) found that only 14.6% of the initial anthocyanins in plums remained after 1 h of drying at 95 °C, whereas 45.6% remained upon drying at 55 °C. Studies have shown that anthocyanins are readily destroyed by heat during food processing (*7*, *40*). Apart from heat, many other factors such as light, temperature, agronomics, and storage, among other variables, are also responsible for the degradation of anthocyanins during the drying and processing of fruits (*6*, *7*). In black currant juice, all anthocyanins disappeared during a 9 weeks of storage at 37 °C, whereas 60% remained after 6 months of storage at 20 °C (*41*). In this study, commercial sun-drying of dates (at 30−50 °C for 7−10 days) led to complete destruction of anthocyanins. Wrolstad (*42*) stated that the degradation of anthocyanins during drying and storage is due to enzymatic and nonenzymatic browning reactions. Several enzymes have been found to be involved in the degradation of anthocyanins; these include glycosidase and polyphenol oxidase (*6*).

Total Carotenoids. Total carotenoid contents in fresh dates of Fard, Khasab, and Khalas were 1.39, 1.31, and 3.03 mg/100 g, respectively (**Table 2**). The highest total carotenoids in Khalas was expected, as this variety has a yellow color, whereas the other two varieties are red. Ben-Amotz and Fishler (*43*) reported that the carotenoid content of freeze-dried date was 0.22 mg/100 g of dry weight (~0.18 mg/100 g of fresh weight). Although this is much lower than those found in the present study, this is probably due to the existing differences between the two samples in variety, maturation, storage, and analysis conditions. Hart and Scott (*44*) surveyed the carotenoid content of vegetables and fruits commonly consumed in the United Kingdom. The total content of carotenoids in eight fruits ranged from 0.017 to 2.263 mg/100 g of fresh weight, being lowest in strawberries and highest in mandarins. Thus, dates can be considered a good source of carotenoids compared to the above fruits.

Similar to anthocyanins, commercial sun-drying caused a significant ($p < 0.05$) loss (ranging from 4 to 29.8%) of carotenoids in date varieties except in Khalas, which was insignificant ($p > 0.05$) (**Table 2** and **Figure 2**). Several studies reported the loss of carotenoids in carrot and orange juices during processing and storage (*45*, *46*). Mahanom et al. (*47*) reported that 64% of carotenoids was lost after drying (at 50 °C for 9 h) in the herbal preparation of eight medicinal plant leaves. In addition, lycopene losses during the processing of tomato paste varied from 9 to 28% (*48*).

A number of mechanisms for the reaction and decomposition of carotenoids in plant materials have been reported. These include enzymatic processes, autoxidation, and thermal degradation (*23*, *49*, *50*). Enzymes such as catalase and peroxidase cause carotenoid degradation during processing. Thermal processing has been shown to convert the all-trans form of β-carotene to cis forms (*51*), and this causes a slight shift in the absorbance maximum of carotenoids. Such isomerization induced by exposure to light and processing treatments results in alteration of carotenoid activity.

Total Phenolics. The mean total content of phenolics ranged from 134 to 280 mg of FAE/100 g and from 217 to 343 mg of FAE/100 g in fresh and sun-dried date varieties, respectively

Table 3. Contents of Free Phenolic Acids (Milligrams per 100 g) in Fresh and Sun-Dried Date Varieties[a]

	Fard		Khasab		Khalas	
phenolic acid	fresh	sun-dried	fresh	sun-dried	fresh	sun-dried
protocatechuic	nd	nd	nd	2.04 ± 0.47	nd	nd
vanillic	2.13 ± 0.01b	3.82 ± 0.08c	1.45 ± 0.02d	2.18 ± 0.02b	1.70 ± 0.03e	4.14 ± 0.47f
syringic	5.49 ± 0.17b	6.02 ± 0.56c	nd	nd	nd	4.52 ± 0.09d
ferulic	4.65 ± 0.17b	4.93 ± 0.02c	1.16 ± 0.01d	1.84 ± 0.19e	4.71 ± 0.69b	5.08 ± 0.32c
total	12.27 ± 0.33b	14.77 ± 1.46c	2.61 ± 0.02d	6.06 ± 0.30e	6.41 ± 0.69f	13.74 ± 0.89g

[a] Data are expressed as mean ± SD ($n = 3$) on a fresh weight basis. The moisture contents of Fard, Khasab, and Khalas were 48.8, 43.6, and 33.4% (fresh) and 18.5, 16.5, and 12.6% (sun-dried), respectively. Means ± SD followed by the same letter, within a row (Fard, Khasab, and Khalas), are not significantly different ($p > 0.05$). nd, not detected.

Table 4. Contents of Bound Phenolic Acids (Milligrams per 100 g) in Fresh and Sun-Dried Date Varieties[a]

	Fard		Khasab		Khalas	
phenolic acid	fresh	sun-dried	fresh	sun-dried	fresh	sun-dried
gallic	0.48 ± 0.06b	1.60 ± 0.02c	nd	nd	nd	3.09 ± 0.16d
protocatechuic	4.49 ± 0.05b	8.34 ± 0.16c	2.32 ± 0.15d	4.44 ± 0.32b	nd	nd
p-hydroxybenzoic	nd	nd	0.49 ± 0.04	nd	nd	nd
vanillic	nd	nd	nd	nd	nd	2.26 ± 0.06
caffeic	nd	nd	nd	nd	10.10 ± 0.60b	7.57 ± 0.45c
syringic	1.70 ± 0.17b	3.22 ± 0.76c	nd	nd	0.17 ± 0.02d	2.57 ± 0.02e
p-coumaric	0.49 ± 0.01b	1.41 ± 0.14c	1.93 ± 0.08d	1.71 ± 0.16e	6.25 ± 0.15f	14.19 ± 0.31g
ferulic	4.00 ± 0.35b	6.08 ± 0.25c	2.06 ± 0.04d	6.11 ± 0.15e	12.27 ± 0.41f	13.28 ± 0.43g
o-coumaric	nd	nd	0.04 ± 0.01b	1.92 ± 0.02c	1.46 ± 0.01d	6.71 ± 0.11e
total	11.16 ± 0.50b	20.65 ± 0.79c	6.84 ± 0.21d	14.18 ± 0.39e	30.25 ± 0.98f	49.67 ± 3.91g

[a] Data are expressed as mean ± SD ($n = 3$) on a fresh weight basis. The moisture contents of Fard, Khasab, and Khalas were 48.8, 43.6, and 33.4% (fresh) and 18.5, 16.5, and 12.6% (sun-dried), respectively. Means ± SD followed by the same letter, within a row (Fard, Khasab, and Khalas), are not significantly different ($p > 0.05$). nd, not detected.

(**Table 2**). Of the varieties studied, Fard had the highest amount of total phenolics in both fresh and sun-dried states. Mansouri et al. (*52*) studied the phenolic profiles of seven different varieties of ripe date fruits grown in Algeria. They found that total phenolic content ranged from 2.49 to 8.36 mg/100 g of fresh weight, expressed as gallic acid equivalents. These levels are much lower compared to those found in this study; use of different phenolic acid standards (ferulic acid and gallic acid, which make the quantitative comparison invalid) or various factors such as variety, growing condition, maturity, season, geographic origin between the two countries, fertilizer, soil type, storage conditions, and amount of sunlight received, among others, might be responsible for the observed differences. In a previous study (*26*), we found that the mean total content of phenolics among two native varieties of cherry laurel and pekmez ranged from 454 to 1444 mg of FAE/100 g of fresh weight. Total phenolic contents of various fresh fruits and their different cultivars have been studied by many researchers (*36*, *53*−*55*). The ranges of total phenolic contents reported were as low as 9.1 mg/100 g of fresh weight in white-flesh nectarines (*55*) and as high as 1790 mg/100 g of fresh-frozen weight in one genotype of *Ribes* L. (black currants) (*36*). In comparison, dates may be considered as a good source of total phenolics.

Total phenolic contents of sun-dried dates were significantly ($p < 0.05$) higher than those of fresh dates in Fard, Khasab, and Khalas by 22.5, 29.9, and 153%, respectively (**Figure 2**). Shahidi and Naczk (*6*) reported that drying, in general, is regarded as unfavorable due to the possibility of inducing oxidative decomposition either enzymatically by polyphenol oxidase and glycosidase or by thermal degradation of phenolic compounds. However, total phenolic contents in dates showed increases after sun-drying, and this could be explained by the degradation of tannins by temperature and maturation enzymes during the drying process, which leads to the release of phenolic compounds (*56*). Thus, the linkages between *p*-coumaric acid and lignin and between ferulic acid and arabinoxylans could be broken at high temperatures.

Phenolic Acids. The content of free and bound phenolic acids in three native fresh and sun-dried date varieties are listed in **Tables 3** and **4**, respectively. Free and bound phenolic acids were analyzed using three different hydrolysis procedures (free, alkaline, and acid) within the same tube. A total of nine phenolic acids were detected, of which five consisted of hydroxylated derivatives of benzoic acid (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, and syringic acid) and four were cinnamic acid derivatives (caffeic acid, *p*-coumaric acid, ferulic acid, and *o*-coumaric acid). In addition to this, there were several unknown compounds in both free and bound phenolic acids. In total, 8, 8, and 10 phenolic acids (free and bound) were tentatively identified and quantified in combination of both fresh and sun-dried dates of Fard, Khasab, and Khalas, respectively.

Four free phenolic acids (protocatechuic acid, vanillic acid, syringic acid, and ferulic acid) were detected in dates, with total concentrations varying between 2.61 and 12.27 mg/100 g in fresh dates, and between 6.06 and 14.77 mg/100 g in their sun-dried counterparts (**Table 3**). Fard had the highest content of free phenolic acids, followed by Khalas and Khasab in both fresh and sun-dried samples. Sun-drying significantly ($p < 0.05$) increased the total concentration of free phenolics in all date varieties. Among the identified free phenolic acids, vanillic acid, syringic acid, and ferulic acid were present in all varieties as the major compounds except for the Khasab variety. In contrast, protocatechuic acid was detected only in sun-dried dates of Khasab variety.

Total bound phenolic was a combination of phenolic acids extracted by both alkaline and acid hydrolyses. Nine bound phenolic acids liberated by alkaline and acid hydrolyses were

successfully identified in fresh and sun-dried date varieties, of which protocatechuic acid and ferulic acid were the predominant bound phenolic acids in Fard and Khasab varieties. However, caffeic acid, *p*-coumaric acid, ferulic acid, and *o*-coumaric acid were the major phenolic acids in the Khalas variety. The total bound phenolic contents were in the ranges of 6.84−30.25 mg/100 g in fresh dates and 14.18−49.67 mg/100 g in sun-dried dates (**Table 4**). The Khalas variety was richest in total bound phenolic acids (both fresh and sun-dried), followed by Fard and Khasab. Sun-drying significantly ($p < 0.05$) increased the total concentration of bound phenolics in all date varieties.

Regnault-Roger et al. (*57*) studied phenolic acids in dried Tunisian dates and found eight phenolic acids (gallic acid, protocatechuic acid, *p*-hydroxybenzoic acid, vanillic acid, caffeic acid, syringic acid, *p*-coumaric acid, and ferulic acid). Although this study showed a similar phenolic acid profile, the concentrations of phenolics was much higher than those reported earlier. Recently, Mansouri et al. (*52*) studied phenolic profiles of seven different varieties of ripe date fruits grown in Algeria and found that all varieties contained *p*-coumaric acid, ferulic acid, and sinapic acid as well as some cinnamic acid derivatives, but these were not quantified. Three different isomers of 5-*o*-caffeoylshikimic acid were also detected. However, the date varieties used in this study were different from those used by Regnault-Roger et al. (*57*) and Mansouri et al. (*52*). Many factors (such as location, environmental characteristics, and fruit maturity) have been reported to influence the content and variability of phenolic compounds within the same fruit type (*37*, *58*, *59*). The contents of free and bound phenolic acids in dates examined in this study were comparable with those previously reported for other fruits (*25*, *26*).

It has been reported that caffeic acid, sinapic acid, ferulic acid, and *p*-coumaric acid are more antioxidative than protocatechuic acid, syringic acid, vanillic acid, and protocatechuic acid (*60*). Because dates were found to be a good source of the more active phenolic acids, they may be considered as good sources of natural antioxidants. Besides their naturally occurring antioxidant properties, phenolic acids can also influence product flavor and color.

Total phenolic acids of sun-dried dates were significantly ($p < 0.05$) higher than those of fresh dates in Fard, Khasab, and Khalas by 51.2, 114.2, and 73%, respectively (**Figure 2**). These values are in good agreement with those of Karadeniz et al. (*61*), who found an increase in oxidized cinnamic acid (quantified as cholorogenic) and protocatechuic acid by 100% after sun-drying of grapes. Unlike this study, Ferreira et al. (*13*) reported a decrease of 96% in bound hydroxycinnamic acids (caffeoylquinic and *p*-coumarylmalic) during sun-drying of pears. They related this decrease to the enzymatic degradation of polyphenols by polyphenol oxidase. Processing, such as drying, malting, fermentation, and storage, generally increases the content of free phenolic acids in fruits by hydrolyzing the bound phenolic acids and releasing them as free acids, consequently giving rise to a general decrease in the content of bound phenolic acids. During processing, complex phenolic compounds, including tannins and lignins, are subjected to hydrolysis (temperature, enzymatic, and nonenzymatic oxidations), which leads to the production of lower molecular weight phenolic compounds such as free and bound phenolic acids (*62*, *63*).

The results presented in this work suggest that date varieties serve as a good source of natural antioxidative compounds that could potentially be used in food and nutraceutical formulations. Date varieties had different levels and patterns of phenolic acids, most of which were present in the bound form. A significant ($p < 0.05$) amount of antioxidants, anthocyanins, and carotenoids was lost during the sun-drying of dates, whereas the total contents of phenolics and phenolic acids (both in free and bound) increased significantly ($p < 0.05$). Although it is difficult to assess, the Khalas variety, which is considered to be of premium quality, had higher antioxidant activity, total carotenoids, and bound phenolic acids than other varieties studied. The observed differences among varieties may relate to the existing differences in their moisture content and/or the sun-drying-induced changes. Although determination on a dry weight basis may overcome the issue of moisture difference, fresh weight calculation was deemed to be more appropriate from a consumption viewpoint, and this clearly represents the nutritional benefits of these fruits. Further research is needed to compare the fresh and sun-dried date varieties (on both fresh and dried weight bases) in terms of their free radical scavenging activities, reducing power, and oxidation of human low-density lipopreotein cholesterol as well as antioxidant activity in a β-carotene−linoleate model system.

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