

Phenolic profile and antioxidant activity of Saudi date palm (*Phoenix dactylifera* L.) fruit of various cultivarsIsmail Hamad*^{1,2}¹Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Aljouf University, Sakaka
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Abstract: Background: The chemical components and antioxidant capacities of three Saudi date palm fruit (DPF) cultivars, named Al Sagey, Helwat Al Jouf and Al Sour were investigated in this study. Methods: Phenolic and flavonoid profiles of the date cultivars were determined using high performance liquid chromatography with diode array detection (HPLC-DAD). Glutathione and ascorbate contents were analyzed by reversed phase HPLC separation, followed by amperometric detection and their redox status were calculated, while antioxidant capacities were evaluated in vitro using scavenging assays of 1,1-diphenyl-2-picrylhydrazyl radical and anti-lipid peroxidation ability. Results & Discussion: The results showed that the tested cultivars possess different antioxidant capacities, and had different phenolic and flavonoid patterns. Al Sagey cultivar possessed the strongest antioxidant capacities and the highest phenolic contents. AL Sagey and Helwat Al Jouf showed comparable glutathione and ascorbate redox status while Al Sour Glutathione redox status was the least. This study was carried out because any information on the health-promoting components of dates will enhance the knowledge and appreciation for the uses of dates in these health-promoting products.

[Ismail Hamad. **Phenolic profile and antioxidant activity of Saudi date palm (*Phoenix dactylifera* L.) fruit of various cultivars.** *Life Sci J* 2014;11(10):1268-1271]. (ISSN:1097-8135). <http://www.lifesciencesite.com>. 188

Key Words: Chemical Composition, Antioxidant capacities, High performance liquid chromatography, Date palm fruits, DPPH.

Introduction

For many centuries, the date fruit (*Phoenix dactylifera* L.) has been considered as the most important components of the diets of people in the Arab world (Vayalil, 2002) and Muslim worldwide believe that consumption of date fruits, particularly in the morning on an empty stomach, can reverse the actions of any toxic material that the subject may have been exposed to.

The total annual world production of date fruits was reported to be 7.52 million tons during the 2009 crop year, and is expected to increase due to recent advances in tissue culture techniques and the improved agricultural practices. Egypt, Iran, Saudi Arabia, the United Arab Emirates, Pakistan, Algeria, Iraq, Sudan and Oman are the main producers (FAO, 2011, <http://faostat.fao.org/>).

The date is a highly nutritious fruits from the Palmaceae family. Dates have higher caloric content and more essential minerals and vitamins than most other fruits. Date palm fruits contain a high percentage of carbohydrates (total sugars, 44-88 %), fat (0.2-0.5 %), protein (2.3-5.6 %), 15 kinds of salts and minerals, vitamins, and a high percentage of dietary fiber (6.4-11.5 %). The flesh of dates contains 0.2-0.5 % oil, while the seed contains 7.7-9.7 % oil. In fact, the weight of the seed is 5.6- 14.2 % of the date (Al-Farsi et al., 2005).

Dates are also important because of their antioxidant and antimutagenic properties. Vayalil (2002) reported that dates have phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones) that provide antioxidant activities and date fruit could inhibit benzo(a)pyrene-induced mutagenicity on *Salmonella* tester strains TA-98 and TA-100 with metabolic activation.

Many studies have confirmed that intake of antioxidants is effective in preventing or suppressing many diseases, therefore, there is a growing interest in natural phenolic antioxidants present in medicinal and dietary plants that might help preventing or decreasing oxidative damages without exerting harmful side effects (Silva et al. 2005).

This study was carried out because any information on the health-promoting components of dates will enhance the knowledge and appreciation for the uses of dates in these health-promoting products.

The objectives of this study were to characterize the major antioxidant related chemical components of 3 date varieties cultivated in Saudi Arabia extract and to evaluate their antioxidant capacities.

2. Materials and Methods**Materials**

Al Sugey, Helwat Al Jouf and Al Sour were the varieties investigated in this study. The 3 cultivars were obtained at “tamr” stage from a well-known commercial date pack house in the Sakaka City, Al

Jouf, Saudi Arabia. All reagents and chemicals were analytical grade and obtained from Sigma Aldrich, St. Louis, USA.

Methods

HPLC analysis of free phenolic and flavonoid compounds

Date fruits were extracted on aqueous acetone, and then were dried under reduced pressure using a rotary evaporator (SENCO, Shanghai Senco Technology Company, Shanghai, China) at 45°C under reduced pressure.

After drying, the residues were dissolved in HPLC grade MeOH to give 1000 mg/l and measured according to (Gomaa and Abdelgawad 2012). 20 µl of methanol-dissolved sample was injected into HPLC system (Shimadzu class, Shimadzu Corporation, Kyoto, Japan). HPLC system consisted of diode-array detector and column Lichrosorb Si-60, 7 µm, 3 × 150 mm.

Mobile phase consisted of water/formic acid, 90:10, v/v; and acetonitrile/water/formic acid, 85:10:5, v/v/v., tentatively identified phenolic acids and flavonoids were quantified with a calibration curve obtained with the corresponding standards. The results were expressed as milligrams per 100 g (mg/100 g DW).

HPLC measurement of ascorbate, glutathione, and their redox status

Reduced ascorbate (ASC), reduced glutathione (GSH) and reduced homo-glutathione (hGSH) were quantified by HPLC analysis according to (Potters et al., 2004).

Total antioxidant concentration (reduced+oxidized) was determined after reduction with 0.04 M DTT for 10 min at room temperature, and the redox status was calculated as the ratio of the reduced form to the total concentration.

DPPH Free Radical Scavenging Assay

The 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging activity of extracts was estimated according to the method explained by Cheung et al. 2003 with some modifications. Aliquots of 160 µl of 0.2 mM DPPH in methanol were mixed with 40 µl of each extract (in a concentration of 0.01–1 mg/ml). The mixtures were left under subdued light for 10 min. The absorbance at 520 nm was measured against blank. The radical scavenging activity was measured as a decrease in the absorbance of DPPH and was calculated using the following equation;

$$\text{Scavenged DPPH} = \left[1 - \frac{(A_{\text{sample}} - A_{\text{sample blank}})}{A_{\text{control}}} \right] * 100$$

Where A_{control} , A_{sample} , and $A_{\text{sample blank}}$ represent the absorbance of the control group (160 µl 0.2 mM DPPH and 40 µl methanol or water), sample group [160 µl 0.2 mM DPPH and 40 µl extract or reference compounds (Gallic acid)], and sample blank (160 µl

methanol and 40 µl extract or reference compounds), respectively.

Anti-Lipid Peroxidation Assay

Lipid peroxidation was carried out as described by Patro et al. 2002 with some modifications. Briefly, small unilamellar vesicles were prepared from phosphatidylcholine (300 mg phosphatidylcholine in 30 mL 10mM phosphate buffer at pH 7.4 were sonicated on ice for 2 h). To a total volume (1 ml) containing potassium phosphate buffer at pH 7.4 (10mM), the liposome (250µl), and extract or extraction solvent (450µl), was added FeCl₂, H₂O₂ and ascorbic acid each in a final concentration of 125µM. After incubating the mixture at 30 °C for 4 h, 250 µL of the final mixture was added to 500µl TCA-TBA-HCl reagent (15% w/v, TCA; 0.375% w/v, TBA; 0.25 M HCl), heated at 100 °C on a boiling water bath for 15 min. After centrifugation at 3,000 g for 5 min the absorbance was monitored at 532 nm against blank.

Statistical Analysis

Experimental analyses were performed in triplicate. Data were recorded as mean ± standard deviation and analyzed by GraphPad prism version 5.

3. Results and Discussion

Antioxidant capacities of date fruit cultivars

Many studies showed that the hepatoprotective, nephroprotective and neuroprotective effects of medicinal plants are related to their antioxidant capacity (Ali and Abdu 2011, Saafi et al 2011 and Pujari et al 2011). To evaluate the antioxidant capacity of various date cultivars the phenolic and flavonoid profiles, the ascorbate, glutathione and the ability to scavenge DPPH and peroxy radical were determined.

Phenolic and flavonoid profiles of date fruit cultivars

Many biological activities of medicinal plants, such as antioxidant, anti-inflammatory and antimicrobial are attributed to their phenolic acids and flavonoids contents (Ren et al. 2003, Stevenson and Hurst 2007). Quantitative analyses of phenolic and flavonoid compounds found in 12 date cultivars were determined using HPLC-DAD techniques and the results are represented in table 1 and 2 respectively. Nine free phenolic acids (Caffeic acid, Ferulic acid, Protocatechuic acid, Catechin, Gallic acid, *p*-Coumaric acid, Resorcinol, Chlorogenic acid and Syringic acid) and five flavonoid compounds (Quercetin, Luteolin, Isoquercetin, Apigenin and Rutin) were determined. A Similar phenolic and flavonoid profiles were described for Algerian (Benmeddour et al. 2012), Tunisian (Chaira et al. 2009) and Omani (Al-Farsi et al. 2005) dates cultivars. Al Sagey cultivar contained the highest amount of total phenolic (28.57 mg /100 g DW) and

Al Sour contained the lowest amount of total phenolic (11.30 mg/100 g DW). Gallic acid was the most dominant phenolic compound in all date cultivars with the highest content was observed in Al Sagey cultivar (20.42 mg/100 g DW).

The obtained results are slightly lower than those reported by **Benmeddour et al. (2012)** who found that gallic acid content varied from 70 to 92.20% in 10 Algerian date cultivars. In contrast, Al-Farsi et al. (2005) found that ferulic acid was the major phenolic acid in date cultivars from Oman. The obtained results shows that Saudi date cultivars used in this study are rich source of gallic acid. This is very significant since gallic acid; a potent antioxidant that possesses antimutagenic and anticarcinogenic activities (**Stich et al 1984**) is well absorbed in the human body (**Shahrzad et al., 2001**).

Al Sagey cultivar contained the highest amount of total flavonoids (3.94 mg/100g DW) followed by Helwat Al Jouf (3.55 mg/100g DW) and Al Sour (1.44 mg/100g DW). Quercetin was the main flavonoid in all date cultivars and represents 37.8% on average with the highest level observed in Helwat Al Jouf cultivar (1.36 mg/100 g DW). **Hong et al., 2006** identified 13 flavonoid glycosides of luteolin, quercetin and apigenin in Deglet Noor date fruit at the khalal stage.

Ascorbate, glutathione, homo-glutathione and their redox status

Ascorbate and Reduced glutathione (GSH) play and important role in defense against oxidative stress as a part of non-enzymatic and well as enzymatic antioxidants (**Møller et al. 2007**).

The ascorbate, glutathione content in three date cultivars were determined and their redox status were calculated (table 3).

The highest level of reduced glutathione was observed in Al Sagey cultivar (0.1 μ mol/g FW) and the lowest content was observed in Helwat Al Jouf cultivar (0.038 μ mol/g FW). Glutathione redox status ranged from 25.057% (Al Sour) to 54.086% (Al

Sagey). The three cultivars showed a comparable ascorbate redox status.

To our knowledge there is no previous report on the ascorbate and glutathione content in date fruits. The same pattern were observed for reduced and redox status of ascorbate.

DPPH Free Radical Scavenging Activity

The 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical-scavenging ability is widely used to evaluate the free radical scavenging capacity of antioxidants (**Sanchez-Moreno 2002**). The DPPH scavenging activities of 100 μ g of the acetone extract of 3 Saudi date cultivars are given in Table 4. The results showed that all tested cultivars had a free radical scavenging ability. Al Sagey had the strongest DPPH scavenging capacity (33%), whereas Al Sour cultivar had the lowest value (17.33%). Our results are lower than **Chaira et al. 2009**, who demonstrated that 100 μ g of Deglet Noor cultivar extract could inhibit DPPH radical formation by 54%.

Anti-lipid peroxidation assay

Lipid peroxidation is considered an important factor in the pathogenesis of age-related neurodegenerative diseases (**Reed 2011**). The results showed that approximately all the tested samples, inhibited lipid peroxidation. However, Al Sagey extract showed the highest scavenging potential while Al Sour showed the least anti-lipid peroxidation activity (Table 4). On the basis of IC₅₀ values pattern of anti-lipid peroxidation the date samples can be arranged as Al Sagey > Helwat Al Jouf > Al Sour. Our results are lower than those of Chaira et al. 2009 found that Tunisian Korkobbi cultivar had the best lipoperoxyl radical-scavenging activity with 83% percentage of inhibition.

The present work showed that the tested date cultivars are a good source of natural antioxidant compounds which make date palm fruits suitable for application in the pharmaceutical field and could be considered as a functional food or functional food ingredient particularly Al Sagey cultivar.

Table 1: Content of individual phenolic compounds of 3 Saudi date cultivars determined by HPLC-DAD (mg/100g DW)

Phenolic compounds									
Caffeic acid	Ferulic ACID	Protocatechuic acid	Catechin	Gallic acid	p-Coumaric acid	Resorcinol	Chlorogenic acid	Syringic acid	total phenolic
Cultivars									
Al Sagey	0.024±0.005	2.561±0.570	0.207±0.046	0.769±0.171	20.417±4.542	3.085±0.869	0.031±0.009	1.205±0.268	28.568±4.784
Helwat Al Jouf	0.019±0.003	1.816±0.102	0.174±0.030	0.580±0.018	15.385±0.467	3.581±0.627	0.037±0.006	0.982±0.172	22.929±0.494
Al Sour	0.013±0.001	1.445±0.091	0.606±0.038	0.262±0.017	6.961±0.441	1.497±0.095	0.015±0.001	0.411±0.026	11.303±0.715

Table 2: Content of individual flavonoid compounds of 3 Saudi date cultivars determined by HPLC-DAD (mg/100g DW)

Flavonoid compounds						
Cultivars						
	Quercetin	Luteolin	Apigenin	Isoquercetrin	Rutin	Total Flavonoid
Al Sagey	1.270±0.358	0.042±0.012	0.390±0.087	0.974±0.217	1.265±0.281	3.940±0.215
Helwat Al Jouf	1.362±0.041	0.045±0.001	0.333±0.047	0.734±0.022	1.081±0.152	3.555±0.134
Al Sour	0.616±0.039	0.020±0.001	0.133±0.008	0.199±0.013	0.431±0.027	1.400±0.089

Table 3: Glutathione and ascorbate contents and their redox status of 3 date fruit cultivars

Cultivars	TGSH ($\mu\text{mol/g FW}$)	GSH ($\mu\text{mol/g FW}$)	GSH Redox status (%)	TASC $\mu\text{mol/gFW}$	ASC $\mu\text{mol/gFW}$	ASC Redox status (%)
Al Sagey	0.186 \pm 0.025	0.100 \pm 0.011	54.086 \pm 1.443	0.368 \pm 0.024	0.372 \pm 0.034	98.922 \pm 2.628
Helwat Al Jouf	0.069 \pm 0.009	0.038 \pm 0.004	54.737 \pm 6.428	0.281 \pm 0.018	0.283 \pm 0.026	99.181 \pm 3.562
Al Sour	0.249 \pm 0.033	0.062 \pm 0.007	25.057 \pm 0.668	0.277 \pm 0.018	0.287 \pm 0.026	96.682 \pm 3.472

Table 4: Antioxidant capacity of 3 Saudi date cultivars

Cultivars	DPPH Scavenging activity	Lipid peroxidation inhibitory activity
	% Inhibition (100 $\mu\text{g/ml}$)	IC50 (mg/ml)
Al Sagey	33.000 \pm 2.000	1.687 \pm 0.076
Helwat AlJouf	32.000 \pm 3.606	1.837 \pm 0.159
Al Sour	17.333 \pm 3.512	2.448 \pm 0.237
Gallic acid	51.082 \pm 3.361	ND

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10/15/2014