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ScienceDirect
Food Chemistry 107 (2008) 1636–1641
Food Chemistry
www.elsevier.com/locate/foodchem

Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran

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Received 7 June 2007; received in revised form 1 October 2007; accepted 10 October 2007

Abstract

Edible parts of date palm (*Phoenix dactylifera*) fruits (DPF) from Iran were analyzed for their antioxidant activities (AA) using Trolox equivalent antioxidant capacity (TEAC) method, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) radical cation ($ABTS^{\bullet+}$) assays and the ferric reducing/antioxidant power method (FRAP assay). The total phenolic content (TPC) and total flavonoid content (TFC) of the DPF were measured using Folin–Ciocalteau and aluminum chloride colorimetric methods, respectively. The samples used included four types of soft dates (SD) namely Honey date, Bam date, Jiroft date and Kabkab date; three types of semi-dry dates (SDD) namely Sahroon date, Piarom date and Zahedi date and one type of dry date (DD) which was Kharak date. The AA (ABTS assay) of the DPF were 22.83–41.17, 47.6–54.61 and 500.33 μmol Trolox equivalents/100 g dry weights (dw) for SD, SDD and DD, respectively. The AA (FRAP assay) per 100 g dw sample were 11.65–20, 19.12–29.34 and 387.34 μmol FRAP for SD, SDD and DD, respectively. The TPC ranged from 2.89 to 4.82, 4.37 to 6.64 and 141.35 mg gallic acid equivalents (GAE)/100 g dw, while TFC ranged from 1.62 to 3.07, 1.65 to 4.71 and 81.79 mg catechin equivalents (CEQ)/100 g dw sample for SD, SDD and DD, respectively. Correlation analyses indicated that there was a linear relationship between AA and the TPC or TFC of DPF. This work demonstrates the potential of Iranian dates as antioxidant functional food ingredients.

Keywords: ABTS; Antioxidant activity; FRAP; Iranian date palm fruit; Total phenolic content and total flavonoid

1. Introduction

Reactive free radicals, such as superoxide anion ($O_2^{\bullet-}$), hydroxyl radical ($^{\bullet}OH$), and peroxyl radical ($ROO^{\bullet}$), are particularly reactive and are known to be a biological product in reducing molecular oxygen (Williams & Jeffrey, 2000). Damage mediated by free radicals results in the disruption of membrane fluidity, protein denaturation, lipid peroxidation, oxidative DNA and alteration of platelet functions (Fridovich, 1978; Kinsella, Frankel, German, & Kanner, 1993), which have generally been considered to be linked with many chronic health problems such as cancers, inflammation, aging and atherosclerosis.

An antioxidant, which can quench reactive free radicals, can prevent the oxidation of other molecules and may, therefore, have health-promoting effects in the prevention of degenerative diseases (Shahidi, 1997). The interest in antioxidants has been increasing because of their high capacity in scavenging free radicals related to various diseases (Silva, Souza, Rogez, Rees, & Larondelle, 2007). In this respect, phytochemicals from fruits have been shown to possess significant antioxidant capacities that may be associated with lower incidence and lower mortality rates of degenerative diseases in human (Javanmardi, Stushnoff, Locke, & Vivanco, 2003). The antioxidant

Abbreviations: AA, antioxidant activity; ABTS, 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt; DD, dry date; DPF, date palm fruit; FRAP, ferric reducing antioxidant power; GAE, gallic acid equivalents evaluated by the Folin–Ciocalteu method; SD, soft dates; SDD, semi-dry dates; TEAC, trolox equivalent antioxidant capacity; TFC, total flavonoid content; TPC, total phenolic content; TPTZ, tripydyltriazine.

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doi:10.1016/j.foodchem.2007.10.033

properties of fruits vary depending on their content of phenolic components and vitamins C and E, carotenoids, flavonoids (Saura-Calixto & Goni, 2006).

Date palm (*Phoenix dactylifera*) fruits (DPF) have been an important component of the diet in most of the arid and semiarid regions of the world. Typically DPF contains carbohydrate (total sugars, 44–88%), fat (0.2–0.5%), protein (2.3–5.6%) dietary fiber (6.4–11.5%), minerals (the percentage of each mineral in dried dates varies from 0.1 to 916 mg/100 g date) and vitamins (such as vitamin C, B1, B2, A, riboflavin and niacin) (Al-Hooti, Sidhu, & Quabazard, 1995; Al-Shahib & Marshal, 2003; Sawaya, Khatchadourian, Khalil, Safi, & Alshalhat, 1982). The antioxidant potentials of Algerian dates have been studied by Mansouri, Embarek, Kokkalou, and Kefalas (2005). The TPC and antiradical efficiency of the Algerian ripe date palm fruits were 2.49–8.36 mg gallic acid equivalents (GAE) per 100 g fresh weight and 0.08–0.22, respectively. Native sun-dried dates' varieties from Oman have been studied by Al-Farsi, Alasalvar, Morris, Baron, and Shahidi (2005a, 2005b) and Al-Farsi et al. (2007). The TPC and AA of Omani dates was 172–246 mg GAE per 100 g and 146–162 μmol Trolox equivalents per g (on a fresh weight basis), respectively (Al-Farsi et al., 2007). Dates are produced in 35 countries worldwide and cultivated on about 2.9 million acres of land. The world production estimate of date in 2004 was 6772,068 metric tonnes, and Iran (14% of world production) is the second major producer after Egypt (17% of world production) (FAO, 2004). With the advent of the field of functional food and nutraceuticals, any information on health-promoting components of dates will enhance the knowledge and appreciation for the uses of dates in these health-promoting products. Therefore the AA of Iranian dates was determined and presented in this paper. Such a study has not been reported previously.

The main objective of this study was to evaluate the AA of methanolic extracts from eight different types of DPF from Iran using the ABTS and FRAP methods. The AA was correlated with the TPC and TFC of DPF that were measured using Folin–Ciocalteau and aluminum chloride colorimetric methods, respectively.

2. Materials and method

2.1. Plant material

Fresh ripe date samples used in the experiments consisted of four types of soft dates (SD) locally known as Honey, Bam, Jiroft and Kabkab dates; three types of semi-dry dates (SDD) locally known as Sahroon, Piarom and Zahedi dates and one type of dry date (DD) locally known as Kharak date. Dates samples from several regions of Iran namely Kerman, Bushehr, Khuzestan, Fars, Yazd, Hormozgan and Semnan were procured at the beginning of the 2006 harvest season. The samples were selected identically in terms of size, colour, ripening stage, without damaged and calamity from a date's distribution center in the capital city of Tehran and were transported in paper bags in refrigerator to Malaysia for the studies. Each date weighed about 7–10 g per fruit and for each extraction, approximately 100 g (~10 dates) of each type of dates was used. Three replicates were carried out and 10 dates were used for each replicate for each type of date. These were properly selected from the main distribution center during different days.

2.2. Chemicals and reagents

The compounds 2,2′-azinobis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), 2,4,6-tripyridyl-*S*-triazine (TPTZ) were supplied from Sigma–Aldrich (St. Louis, MO, USA), $FeCl_3 \cdot 3H_2O$, potassium persulphate, sodium acetate, and sodium carbonate were obtained from Sigma–Aldrich. Folin–Ciocalteu reagent, Trolox (6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid) were purchased from Merck. All Chemicals and reagents used were of analytical grade.

2.3. Extraction

The edible part of DPF (100 g) was pitted, crushed and cut to small pieces with a sharp knife and dry-blended for 3 min with a blender (Panasonic, Penang, Malaysia). The DPF was then extracted with 300 ml methanol–water (4:1, v/v), at room temperature (20 °C for 5 h using an orbital shaker. The extracts were then filtered and centrifuged (Hettich Zentrifugen, Tuttlingen, Germany) at 4000*g*, for 10 min and the supernatant was concentrated under reduced pressure at 40 °C for 3 h using a rotary evaporator (IKA- WERKE- RV06ML) to obtain the DPF methanolic crude extract. The crude extract was kept in dark glass bottles for three days inside the freezer until use. The storage conditions (time and temperature) were the same for all types of fruits.

2.4. ABTS assay

Antioxidant activity (AA) was measured using an improved ABTS method as described by Cai, Luo, Sun, and Corke (2004) and Re et al. (1999). The ABTS radical cation ($ABTS^{\bullet+}$) solution was prepared through the reaction of 7 mM ABTS and 2.45 mM potassium persulphate, after incubation at 23 °C in the dark for 16 h. The $ABTS^{\bullet+}$ solution was then diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.005 at 734 nm. $ABTS^{\bullet+}$ solution (3.9 ml; absorbance of 0.700 ± 0.005) was added to 0.1 ml of the test sample and mixed vigorously. The reaction mixture was allowed to stand at 23 °C for 6 min and the absorbance at 734 nm was immediately recorded. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 15 μM) in 80% ethanol. The absorbance of the reaction samples was compared to that of the Trolox standard and the results were expressed in terms of Trolox equivalents (Re et al., 1999).

2.5. FRAP assay

The AA of DPF extracts was determined using a modified method of the assay of ferric reducing/antioxidant power (FRAP) of Benzie and Strain (1999). The FRAP reagent contained 2.5 ml of a 10 mM tripydyltriazine (TPTZ) solution in 40 mM HCl plus 2.5 ml of 20 mM $FeCl_3 \cdot 6H_2O$ and 25 ml of 0.3 M acetate buffer at pH 3.6. Freshly prepared FRAP reagent (3.0 ml) were warmed at 37 °C and mixed with 40 μl of DPF extract and the reaction mixtures were later incubated at 37 °C. Absorbance at 593 nm was read with reference to a reagent blank containing distilled water which was also incubated at 37 °C for up to 1 h instead of 4 min, which was the original time applied in FRAP assay. Aqueous solutions of known Fe (II) concentrations in the range of 100–2000 μM ($FeSO_4 \cdot 7H_2O$) were used for calibration (Shui & Leong, 2006).

2.6. Total phenolic content (Folin–Ciocalteau assay)

Total phenolics were determined using Folin–Ciocalteau reagents (Singleton & Rossi, 1965). DPF extract (40 μl) or gallic acid standard were mixed with 1.8 ml of Folin–Ciocalteu reagent (prediluted 10-fold with distilled water) and allowed to stand at room temperature for 5 min, and then 1.2 ml of sodium bicarbonate (7.5%) was added to the mixture. After standing for 60 min at room temperature, absorbance was measured at 765 nm. Results were expressed as mg gallic acid equivalents (GAE)/100 g sample (Shui & Leong, 2006).

2.7. Total flavonoids

The determination of flavonoids was performed according to the colorimetric assay of Kim, Jeong, and Lee (2003). Distilled water (4 ml) was added to 1 ml of DPF extract. Then, 5% sodium nitrite solution (0.3 ml) was added, followed by 10% aluminum chloride solution (0.3 ml). Test tubes were incubated at ambient temperature for 5 min, and then 2 ml of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 ml with distilled water. The mixture was thoroughly vortexed and the absorbance of the pink colour developed was determined at 510 nm. A calibration curve was prepared with catechin and the results were expressed as mg catechin equivalents (CEQ)/100 g sample.

2.8. Statistical analyses

Different statistical techniques such as analysis of variance (ANOVA), Duncan's multiple range method, Pearson's correlation, and regression analysis were carried out for analyzing the data obtained from different types of dates, and to study the relationship between AA, TPC and TFC. Each parameter was measured twice and doubled checked. SPSS was used to perform the statistical analysis. Data were reported as means ± standard deviation of the mean. Differences at $P < 0.05$ were considered statistically significant.

3. Results and discussion

Dates ripen in four stages, which are known throughout the world by their Arabic denominations; kimri (unripe), khalal (full-size, crunchy), rutab (ripe, soft) and tamr (ripe, reduced moisture). The date goes from one extreme of moisture content of 85% at early Kimiri stage to 50–60% for Khalal, about 35–40% for Rutab, and about 20% for Tamr. Due to variety and growth conditions, DPF vary in shape, size, weight and moisture content. The practical sub-division of DPF into soft date (SD), semi-dry date (SDD) and dry date (DD) is based on their external qualities of texture, pliability and the ratio between glucose, fructose and sucrose content at the tamr stage. Moisture and fiber content are expected also to play a role in determination whether a date is SD, SDD or DD (Ahmed & Ramaswamy, 2006; Shekari & Rajabalian, 2004). Myhara, Al-Alawi, Karkalas, and Taylor (2000) reported that phenolic substance (referred to generically as tannins) were high in the inedible Kimri stage of dates and declined progressively as the dates matured to Tamr stage.

Eight varieties of DPF from Iran used in this study were soft dates (SD) namely Honey, Bam, Jiroft and Kabkab dates (moisture content 18–24%), semi-dry dates (SDD) namely Sahroon, Piarom and Zahedi dates (moisture content 13–15%) and dry date (DD) which was Kharak date (moisture content 8%).

ABTS assay is based on the antioxidant ability to react with $ABTS^{\cdot+}$ radical cation generated in the assay system. In contrast, the ferric reducing antioxidant power (FRAP) assay measures the reduction of ferric iron (Fe^{3+}) to ferrous iron (Fe^{2+}) in the presence of antioxidants, which are reductants with half-reaction reduction potentials above Fe^{3+}/Fe^{2+}. Both methods are widely used to evaluate antioxidant activity in foods and biological systems (Meyer, Frankel, & Lester, 2001).

The averages of AA of DPF based on ABTS assay and FRAP assay are given in Table 1. Kharak date showed the highest level of AA of DPF based on ABTS assay (500.33 μmol Trolox equivalents/100 g dw) and FRAP assay (387.34 μmol FRAP/100 g dw). Jiroft date exhibited the lowest level of AA of DPF based on ABTS assay (22.83 μmol Trolox equivalents/100 g dw), while Kabkab showed the lowest level of DPF based on FRAP assay (11.65 μmol FRAP /100 g dw). The order of AA of DPF based on ABTS assay was: Jiroft < Bam < Kabkab < Honey < Sahroon < Piarom < Zahedi < Kharak, and the order of AA of DPF based on FRAP assay was: Kabkab < Jiroft < Bam < Zahedi < Honey < Sahroon < Piarom < Kharak. Analysis of variance (ANOVA) showed strong difference between all types of dates since the $p < 0.0001$, indicating that the types of dates are different based on the AA. Further analysis was done to investigate

Table 1
Antioxidant activity, total phenolic content and total flavonoid of different date varieties from Iran (based on dry weight)

Variety name	Antioxidant activity		Total phenolic content (mg GAE/100 g dw)	Total flavonoid (mg CEQ/100 g dw)
	TEAC (μmol Trolox equivalents/100 g dw)	FRAP (μmol/100 g dw)		
SD				
Jiroft	22.83 ± 1.49^{a}	13.32 ± 0.83^{a}	2.89 ± 0.04^{a}	1.67 ± 0.18^{a}
Bam	29.61 ± 1.29^{a}	16.0 ± 1.12^{b}	3.49 ± 0.20^{ab}	2.79 ± 0.25^{a}
Kabkab	34.42 ± 1.31^{a}	11.65 ± 0.88^{a}	3.25 ± 0.26^{ab}	1.62 ± 0.20^{a}
Honey	41.17 ± 1.79^{a}	20.0 ± 1.87^{c}	4.82 ± 0.09^{abc}	3.07 ± 0.16^{a}
SDD				
Sahroon	47.60 ± 3.04^{a}	26.93 ± 1.96^{d}	6.64 ± 0.08^{c}	1.65 ± 0.52^{a}
Piarom	53.34 ± 6.19^{a}	29.34 ± 2.03^{d}	6.09 ± 0.20^{bc}	4.71 ± 0.61^{a}
Zahedi	54.61 ± 5.19^{a}	19.12 ± 0.58^{c}	4.37 ± 0.10^{abc}	3.26 ± 0.38^{a}
DD				
Kharak	500.33 ± 47.17^{b}	387.34 ± 1.94^{e}	141.35 ± 4.28^{d}	81.79 ± 14.27^{b}

Values are mean ($n = 3$) ± SD. Values with the same superscript letter are not statistically different at the 5% level. GAE, gallic acid equivalents. CEQ, catechin equivalents.
SD, soft dates; SDD, semi-dry dates; DD, dry date.

the differences between the means using Duncan's multiple range. It was evident that the difference between AA of kharak date with other types of dates was significant ($P < 0.05$).

The TPC of DPF varied from 2.89 to 141.35 mg gallic acid equivalents (GAE)/100 g dw sample. The highest TPC was obtained in kharak date and the lowest TPC was found in the Jiroft date. The order of TPC of DPF is: Jiroft < Kabkab < Bam < Zahedi < Honey < Piarom < Sahroon < Kharak (Table 1).

These results showed that the DPF of Iran had a similar level of phenolic content compared with those of Algerian date palm fruits (Mansouri et al., 2005). The only exception was Kharak date that showed the highest TPC that was several folds higher than other dates. However, Al-Farsi et al. (2007) reported TPC values between 172 and 246 mg gallic acid equivalents/100 g fresh weight of Omani dates, that were closer to the TPC value of Kharak date. It has been suggested that date fruit may contain a higher level of TPC among other fresh and dried fruits (Wu et al., 2004). The TPC values of two date varieties studied by Wu et al. (2004) were 572–661 mg gallic acid equivalents/100 g fresh weight which were higher than those reported by Mansouri et al. (2005) and Al-Farsi et al. (2007).

Total flavonoid content (TFC) of DPF was measured using aluminum chloride colorimetric methods (Table 1). The results showed that the TFC of DPF varied considerably from 1.62 to 81.79 mg in terms of catechin equivalents/100 g dw of sample. The order of TFC of DPF was: Kabkab < Sahroon < Jiroft < Bam < Honey < Zahedi < Piarom < Kharak. The difference between TFC of kharak date with other types of dates was significant ($P < 0.05$) as exhibited by Duncan's multiple range. Even though it is apparent that the flavonoids were an important phenolic compounds contributing to the AA of DPF, it is also possible that other phenolic compounds could also contribute to the antioxidant properties of these types of dates.

A correlation analysis was performed on the AA analysis methods (results not shown) for all DPF samples. The correlation between ABTS and FRAP assays was 0.996, which indicates highly significant ($P < 0.001$).

The two assays of antioxidant capacity used in this study were spectrophotometry-based methods. The differences in the AA measurements among assays could be expected, as each assay has a different mechanism of action or different reaction conditions. ABTS is a method based on reduction of the 2,2′-azinobis (3-ethylbenzothiazoline sulphonate) radical. Although both $ABTS^{\cdot+}$ and FRAP have been widely used to measure the antioxidant capacities of natural extracts based on their ability to reduce the radical cation, the reactions of $ABTS^{\cdot+}$ with free radical scavengers present in the test sample occur rapidly and can be assessed by following the decrease in the sample absorbance at 734 nm. The reaction time of the improved ABTS assay is only 6 min, while the FRAP assay measures the reducing capability by increased sample absorbance based on the ferrous ions released. Therefore the assay may not be complete even for several hours after the reaction started, such that a single end-point of the reaction cannot be determined (Prior, Wu, & Schaich, 2005). Ou, Huang, Hampsch-Woodill, Flanagan, and Deemer (2002) also noted that the FRAP assay has some drawbacks, such as interference, reaction kinetics, and quantitation methods.

TEAC method can measure the AA determined by the decolorization of the $ABTS^{\cdot+}$ through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. The extent of inhibition of the absorbance of the $ABTS^{\cdot+}$ is plotted as a function of concentration in order to determine the TEAC, which can be assessed as a function of time (Fig. 1). The percentage of inhibition was calculated using the equation below:

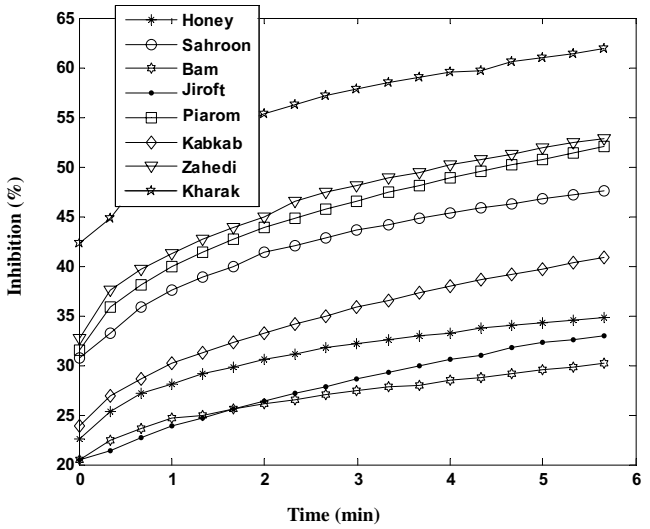

Fig. 1. ABTS radical inhibition of SD, SDD and DD during the reaction time.

$$\text{Inhibition}\,(\%) = \left(\frac{A_{734}^{\text{control}} - A_{734}^{\text{extract}}}{A_{734}^{\text{control}}}\right) x100 \qquad (1)$$

The extent of decolorization is calculated as percentage reduction of absorbance, and this is determined as a function of concentration and calculated relatively to the equivalent trolox concentration.

The antioxidant capacity of DPF can be related to its phenolic content (Mansouri et al., 2005). Therefore regression analysis of AA (ABTS assay) on TPC ($y = 25.42 + 3.36x$) and regression analysis of AA (FRAP assay) on TPC ($y = 3.14 + 0.32x$) of the DPF was performed. It can be seen that TPC showed a high influence on AA (ABTS assay) since the $R^2 = 0.99$ indicating that most of the changes in AA (ABTS assay) belongs to TPC. A high significant influence was also exhibited between TPC and AA (FRAP assay) since the $R^2 = 0.99$. This confirmed that phenolic compounds were the dominant phytochemicals in Iranian DPF that are antioxidative. The role of flavonoids on the antioxidative potentials of DPF was also investigated by performing flavonoids analysis on all dates. The regression analysis of TPC on TFC showed that TFC influences highly the TPC since the $R^2 = 0.99$ ($y = -0.11 + 1.73x$).

It is apparent that the flavonoids were the dominant phenolic compounds of the DPF. This is in agreements with the results reported by Mansouri et al. (2005), which indicated that polyphenolic compounds were the major antioxidants in Algerian date fruits.

The antioxidant activity of dates may be due mainly to the presence of water-soluble compounds with potent free radical-scavenging effects, such as phenolic compounds (mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones) (Mansouri et al., 2005; Vayalil, 2002). Various factors such as variety, growing condition, maturity, season, geographic origin, fertilizer, soil type, storage conditions and amount of sunlight received, among others, might be responsible for the observed differences (Al-Farsi et al., 2007).

4. Conclusions

The antioxidant activities (AA) of Iranian dates were determined and presented in this paper. The total phenolic content (TPC) and total flavonoid content (TFC) of the DPF were measured using Folin–Ciocalteau and aluminum chloride colorimetric methods, respectively. Eight selected date fruits from Iran were examined for AA, TPC and TFC. Dry date's variety had the highest AA, TPC and TFC as compared to those of other dates. A strong correlation existed between AA and TPC and TFC of the dates. This study confirmed the antioxidant potentials of dates from Iran.

Acknowledgements

The authors would like to acknowledge the financial assistance provided by the Universiti Sains Malaysia, Penang. Scientific advice from Professor Baharudin Saad is gratefully acknowledged.

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