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ORIGINAL ARTICLE

Study of phenolic compound and antioxidant activity of date fruit as a function of ripening stages and drying process

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Abstract Edible parts of two varieties of date palm (Mazfati and Kalute varieties) (*Phoenix dactylifera*) fruits (DPF) from Iran were analyzed to determine their phenolic compound and antioxidant activities (AA). Antioxidant activity evaluated using typical methods such as 2, 2-diphenyl-1-picrylhydrazyl (DPPH), reducing power and total antioxidant method. The total phenolic content (TPC) of the DPF was measured using Folin–Ciocalteau method. The samples used in this study included samples were gathered at three stages of khalaal, rutab, tamr and dried date from Bam and Jiroft date. The TPC ranged from 2.89 to 4.82, 1074 to 856.4 and 782.8 mg gallic acid equivalents (GAE/100 gdw sample) for khalal, rutab and tamr stage of Mozafati variety, respectively. This work demonstrates the potential of Iranian dates as good sources of antioxidant which can be used as functional food ingredients. The influence of sun drying process and oven drying at temperature ranged 50–80 °C on phenolic compounds and AA of date palm fruits were investigated. Result of drying process showed that TPC and AA varied with temperature and decreased by increase of drying temperature (from 667.3 to 610.5 mg galic acid in sun dried dates of Mozafati and Kaluteh respectively to 314.2 and 210.4 in dried dates (80 °C) of Mozafati and Kaluteh respectively).

Keywords Iranian date palm fruit · Antioxidant activity · Reducing power · DPPH · Total phenolic content · Total anti oxidant capacity

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Introduction

The role of antioxidants in minimizing or preventing the risk of many human diseases is well recognized (Benzie 2003). Such role is, currently, attracting an increasing attention from various disciplines throughout the world. Databases and recommended daily allowance for antioxidants have been promoted by such interest (Wu et al. 2004).

Generally, dietary plants and plant products are rich sources for natural phytochemical antioxidants including vitamins (ascorbic acid, vitamin A and α-tocopherols), carotenoids and phenolic compounds (Rice-Evans et al. 1997; Demmig-Adams and Adams 2002). The recent explosion of interest in the bioactivity of the flavonoids of higher plants is due to the potential health benefits of these polyphenolic compounds as important dietary constituents (Rice-Evans et al. 1995). Fruits and vegetables have been implicated in preventing or reducing the risk of coronary heart diseases (Rimm et al. 1996), cancer (Block et al. 1992; Donaldson 2004) and other chronic diseases (Cooper 2004). For these reasons, recommendations to increase the dietary intakes of fruits and vegetables have been suggested by many world authorities.

The fruit of the date palm (*Pheonix dactylifera*) is an important commercial crop in the Middle Eastern countries. Date fruits are still considered by many people in this part of the world as a staple food (Sawaya et al. 1982). Date palm is a good source of energy, vitamins, and a group of elements like phosphorus, iron, potassium and a significant amount of calcium (Anwar-Shinwary 1987). The nutritional and biochemical aspects of date fruits were reported by many workers (Al-Farsi et al. 2005; Myhara et al. 2000). They are rich in simple sugars such as glucose and fructose (65–80 %), and a good source of fibers and some essential minerals, but low in fat and protein with no starch (Myhara et al. 2000). Besides nutritional value, date fruits are rich in phenolic compounds that have in vitro antioxidant and antimutagenic properties (Vayalil

2005; Osman et al. 2012; Gao et al. 2011). Date palm fruit development takes place through basically 4 stages named by their Arabic denominations, kimri, khalaal, rutab and tamr. Recently, several studies have reported such activity of date fruits from Algeria (Mansouri et al. 2005), Kuwait (Vayalil 2002), Oman (Al-Farsi et al. 2005) and the USA (Vinson et al. 2005); These studies showed that fresh and dried dates varied quantitatively and qualitatively in their phenolic acids content. Such variations are a reflection of the diversity of date cultivars. More information about the antioxidant activity of various date cultivars at different maturity stages and the relationship of such activity with chemical constituents is needed.

The aim of the present study was evaluation of the potential antioxidant activity and also estimation of the phenolic content at three ripening stages and dried fruit (sun dried and oven dried).

Materials and method

Plant material

Two varieties of date were used in this study, Jiroft Kalute date and Bam Mozafati date that are grown mostly in Kerman Province of Iran. The samples were selected identically in term of ripening stages. The dates were obtained from Jiroft distribution centre at different ripening stage.

Chemicals and reagents

2, 2-diphenyl-1-picrylhydrazyl (DPPH), trichloro acetic acid (TCA), gallic acid, sodium carbonate, Folin–Ciocalteu's phenol reagent, methanol, phosphate buffer, potassium ferricyanide, sulphuric acid, sodium phosphate and ammonium molybdate were purchased from Merck (Darmstadt, Germany); all chemicals were of reagent grade.

Drying of dates

Fresh matured date palm fruits were weighed and dried in cross flow air oven drier (Model OV-160) at different temperatures (50 °C, 60 °C, 70 °C, and 80 °C) and air velocity of 1.5 m^2/s for 48 h in same orientation of product (Falade and Abbo 2006). Drying process started when the drier reached to constant temperature. Dry weight was determined according to the AOAC (1990) method. For preparation of Sun dried samples, date fruit samples were keep in subject to sun (at average temperature 25–45 °C) on wood plate, for 1 week.

Extraction of antioxidants from date fruit

The flesh part of date (100 g) was pitted, crushed and cut to small pieces with a sharp knife and dry-blended for 3 min with a domesticated blender (Panasonic, Penang, Malaysia). The extraction solvent was 300 ml methanol–water (4:1 v/v), and extraction carried out at ambient temperature (20 °C) for 24 h using a laboratory shaker. The ratio of methanol and water which lead to the highest yield of phenolic compounds and flavonoids during preliminary trials selected as best ratio. Similar ratio of methanol to water was used by and biglari et al. (2008). Each extract was filtered with whatman No. 1 filter paper. The obtained filtrate evaporated to dryness at 40 °C in a rotary evaporator (Buchi Laborator). Then all the extracts were dried by a freeze dryer and dried sample constituents stored at 4 °C until use (Arabshahi-Delouee and Urooj 2007)

Estimation of total phenolics

Total phenolic content of each extract was determined by the Folin–Ciocalteu micro method (Slinkard, and Singleton 1977). Briefly, 20 µl of extract solution were mixed with 300 µl of Na2CO3 solution (20 %), then 1.16 ml of distilled water and 100 µl of Folin–Ciocalteu reagent added to mixture after 1 min and 8 min respectively. Subsequently, the mixture was incubated in a shaking incubator at 40 °C for 30 min and its absorbance was measured at 760 nm. Gallic acid was used as a standard for calibration curve. The phenolic content was expressed as gallic acid equivalents by using the following linear equation were obtained from calibration curve:

$$\begin{aligned} A &= 0.98\ C + 9.321 \times 0.001 \\ R^2 &= 0.9965 \end{aligned} \quad (1)$$

Where A is the absorbance and C is concentration as gallic acid equivalents (µg/ml).

Reducing power assay

The ability of extracts to reduce iron (III) was assessed by the Yildirim et al. (2000) method. The dried extract (125–1000 µg) in 1 ml of the corresponding solvent was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of potassium ferricyanide ($K_3Fe(CN)_6$; 10 g/1), then the mixture was incubated at 50 °C for 30 min. After incubation, 2.5 ml of trichloroacetic acid (100 g/1) were added and the mixture was centrifuged at 1650 g for 10 min. Finally, 2.5 ml of the supernatant solution were mixed with 2.5 ml of distilled water and 0.5 ml of $FeCl_3$ (1 g/1) and the absorbance was measured at 700 nm. High absorbance indicates high reducing power.

DPPH radical scavenging activity

The ability of extracts to scavenge DPPH radicals was determined according to the Blois (1958) method. Briefly, 1 ml of a 1 mM methanolic solution of DPPH was mixed with 3 ml of

extract solution in methanol (containing 50–400 μg of dried extract). The mixture was then homogenized vigorously and left for 30 min in the dark place (at room temperature). Its absorbance was measured at 517 nm and activity was expressed as percentage of DPPH scavenging relative to control using the following equation:

$$\text{DPPH scavenging activity}(\%) = \frac{\text{Absorbance of control}--\text{Absorbance of smple}}{\text{Absorbance of control}} \times 100 \quad (2)$$

Total antioxidant capacity

This assay is based on the reduction of Mo (VI) to Mo (V) by the sample and the subsequent formation of a green phosphate/Mo (V) complex at acidic pH (Prieto et al. 1999). An aliquot of 0.1 ml of sample solution (containing 100–500 μg of dried extract in corresponding solvent) was combined in an Eppendorf tube with 1 ml of reagent solution (0.6 M sulphuric acid, 28 mM sodium phosphate, and 4 mM ammonium molybdate). The tubes were capped and incubated in a thermal block at 95 °C for 90 min. the absorbance was measured at 695 nm against a blank when the samples had cooled to room temperature. A typical blank solution contained 1 ml of reagent solution and the appropriate volume of the same solvent used for the sample, and it was incubated under the same conditions as the rest of the samples.

Statistical analysis

All these experiments were replicated three times (from 3 different batches of samples), and the average values are reported. The effect of ripening stages and drying process on antioxidant activity of two date varieties were determined using the analysis of variance (ANOVA) method, and significant differences of means were compared using Duncan's test at 5 % significant level using the SAS software (2001) program.

Result and discussion

Total phenolic compound (TPC)

Date 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. Some of study reported that phenolic substance (referred to generically as tannins) were high in the inedible Kimri stage of date and declined progressively as the date matured to Tamr stage (Ahmad and Ramaswamy 2006; Zhu et al. 2002). The averages of total phenolic compound of DPF based on Folin-Ciocalteau method were shown in Fig. 1(a).

As can be seen from Fig. 1(a) date varieties have significant differences ($P<0.05$) in total phenolic content. Among studied varieties, Mozafati contained the higher amount of total phenolic in comparison with Kalute. These results showed that date palm fruit grown in kerman had a similar level of phenolic content with those of Tunisia date palm fruit (Saafi et al. 2008) and Oman date palm fruit and also with those of Bahrain dates (Allait 2008). However, Mansouri et al. (2005) and Biglari et al. (2008) reported that total phenolic content of Algerian and Iranian date palm fruit ranged from 2.49 to 8.36 mg GAE/100 g of fresh weight and from 2.89 to 6.64 mg GAE/100 g of dry weight respectively. These levels are much lower than this study, except for Kharak date (Iranian dry date) that showed an average of 141.35 mg GAE/100 g dry weight. In the other way, the study reported by Wu et al. (2004), on lipophilic and hydrophilic antioxidant capacities of common foods in the United States found that Deglet Noor and Medjool varieties presented a high level on total phenolic content (661 and 572 mg of GAE per 100 g fresh weight respectively) as compared to our study. Various factors such as variety, growing condition, maturity, season, geographic origin between the two countries, fertilizers, soil type, amount of sunlight received and experimental conditions (storage, extraction) among others might be responsible for the observed differences. The order of total phenolic content of date samples is:

Sun dried date < tamr stage < rutab stage < khalal stage

About variation of TPC in ripening stages, Myhara et al. (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.

As can be seen from Fig. 1(b), date varieties and drying temperatures had significant effect on ($P<0.05$) total phenolic content. By increasing in drying temperature, total phenolic compound decreased. No result was reported about the effect of drying temperature on TPC but Allait (2008) showed that dried date containing lower TPC than fresh date.

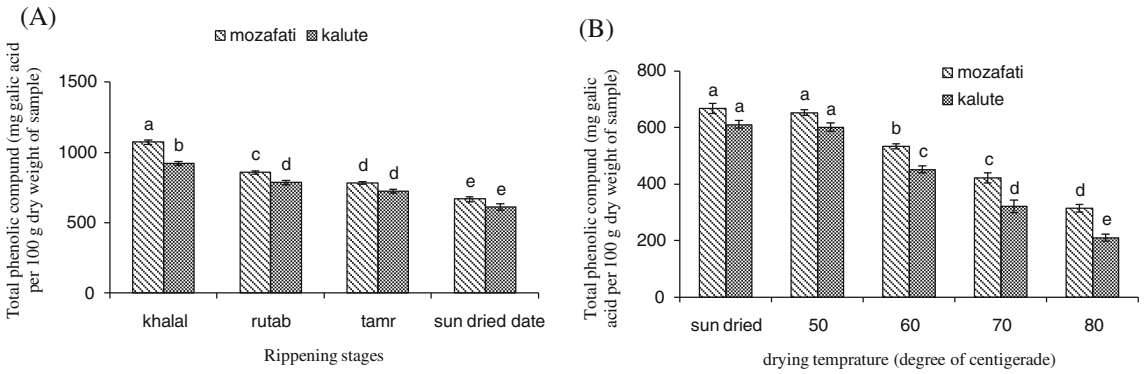

Fig. 1 Effect of **(a)** Ripening stage and sun drying and **(b)** Drying temperature on total phenolic compound of two DPF (Date Palm Fruits) varieties. Each observation is a mean ± SD of 3 replications In each figures means with same superscripts had no significant difference with each other ($P>0.05$)

Antioxidant activity

Total antioxidant activity

In the phosphor molybdenum assay, which is a quantitative method to evaluate water-soluble and fat-soluble antioxidant capacity (total antioxidant capacity), the three extracts concentration exhibited some degree of activity in a dose-dependent manner. The results of total antioxidant capacity of date during ripening stages were shown in Fig. 2(a). As it can be seen from Fig. 2(a), in this study, total antioxidant capacity decreased during rippening stages and increased with increase of extract concentration.

Figure 3(a) showed the effect of drying process on the total antioxidant capacity of dried date in 500 ppm concentration. Results showed that total antioxidant capacity was decreased with increase of drying temperature and no significant differences ($P<0.05$) observed between total antioxidant capacity of sun dried date and oven dried date (oven in 50 °C).

Reducing power assay

The results of reducing power of extracts in ripening stages were shown in Fig. 2(b). Different studies have indicated that the electron donation capacity (reflecting the reducing power) of bioactive compounds is associated with antioxidant activity (Siddhuraju et al. 2002; Yen et al. 1993). In this assay, the ability of extracts to reduce iron (III) to iron (II) was determined.

In this study extracts reducing power were showed significant differences ($p<0.05$) from khalal stage to rutab stage but no significant differences ($p<0.05$) in rutab stage and tamr stage were observed. Khalal stage containing the highest

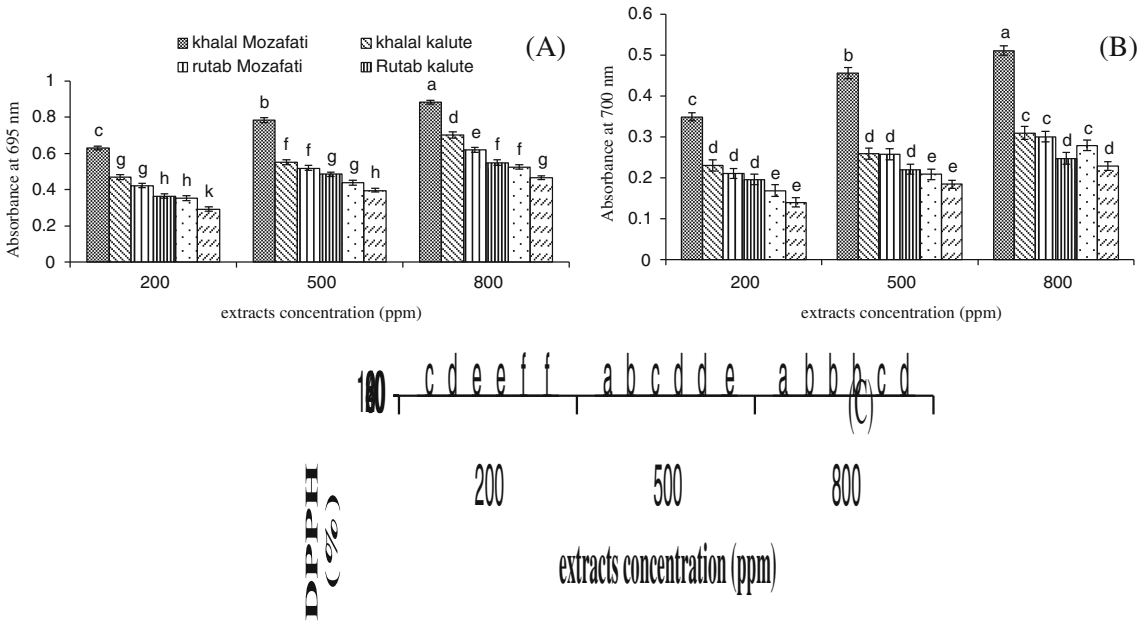

Fig. 2 Changes in **(a)** Total antioxidant capacity, **b** Reducing power and **(c)** DPPH radical scavenging activity of different date extracts during ripening stages Each observation is a mean ± SD of 3 replications In each figures means with same superscripts had no significant difference with each other ($P>0.05$)

amount of total phenolics, was the most potent reducing agent, whereas tamr stage containing the least amount of phenolics, was the weakest in the reducing activity. Similar relations between iron (III) reducing activity and total phenol content have been reported in the literature (Benzie and Szeto 1999; Gao et al. 2000a, b); however the correlation may not be always linear (Yildirim et al. 2000).

The results of the effect of drying temperature on reducing power of dried dates in concentration of 500 ppm were shown in Fig. 3(b).

As can be seen from Fig. 3(b) reducing power of date extracts decreased by increase of drying temperature and no significant differences ($P<0.05$) observed between sun dried date and oven (50 °C) dried date in case of reducing power. Mozafati had higher reducing power than kalute ($p<0.05$). Arabshahi-Delouee and Urooj (2007) reported similar results about the effect of temperature on antioxidant reduction of mulberry (*Morus indica L.*) leaves.

DPPH radical scavenging activity

The results of DPPH radical scavenging activity of extracts affected by ripening stages were shown in Fig. 2(c). Free radicals which are involved in the process of lipid peroxidation are considered to play a major role in numerous chronic pathologies, such as cancer and cardiovascular diseases among others (Dorman et al. 2003). The DPPH radical has been widely used to evaluate the free radicals scavenging ability of various natural products and has been accepted as a model compound for free radicals originating in lipids (Porto et al. 2000).

As it can be seen from Fig. 2(c), Khalal stage that contained the highest amount of total phenolics, was found to be the most active radical scavenger followed by rutab stage and tamr stage. A high correlation between free radical scavenging and the phenolic contents has been reported for fruits (Gao et al. 2000a, b; Jimenez-Escrig et al. 2001; Arabshahi Delouee and Urooj 2007). Figure 2(c) also showed that mozafati date had higher DPPH (%) than kalute variety in all ripening stage ($p<0.05$). The results of DPPH radical scavenging activity of dried date extracts (500 ppm) were shown in Fig. 3(c). This figure showed that DPPH radical scavenging decreased by increasing drying temperature and no significant differences ($P<0.05$) were found between DPPH radical scavenging of sun dried date and dried date with oven (50 °C). These results may be due to reduction of phenolic compound by temperature (Arabshahi Delouee and Urooj 2007).

Conclusions

The antioxidant activities (AA) and total phenolic content (TPC) of Kerman dates were determined and presented in this paper. Two date variety had significant difference in case of antioxidant activity and total phenolic content ($p<0.05$) and mozafati variety had higher that kalute in these case. In both varieties antioxidant activities (AA) and total phenolic content (TPC) decreased by ripening stages and Khalal stage

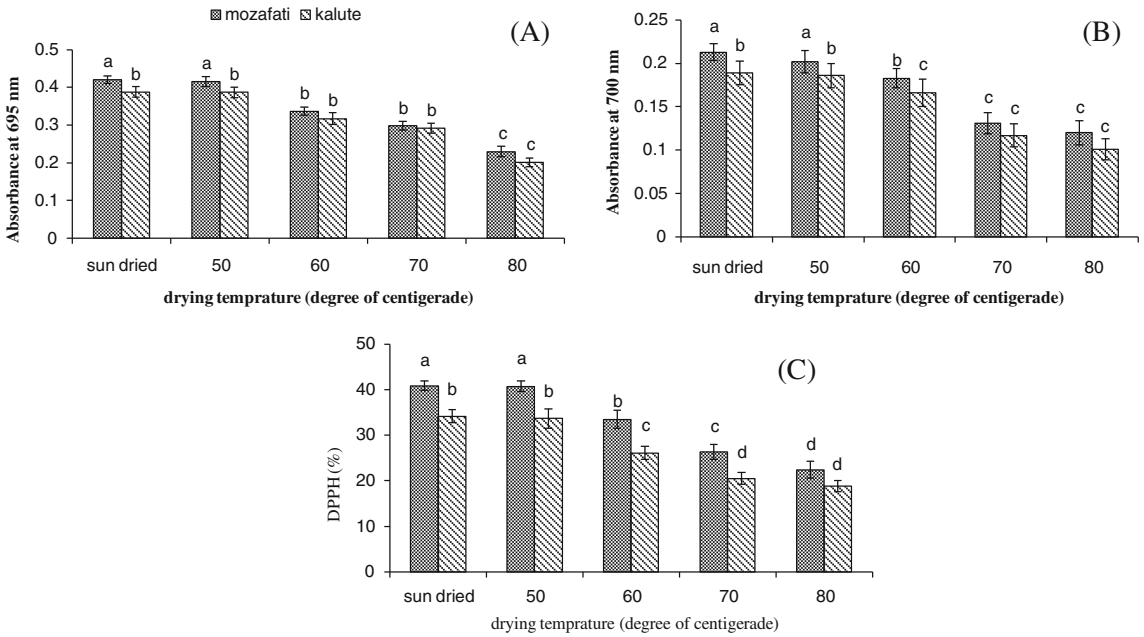

Fig. 3 Effect of drying process on **(a)** Total antioxidant capacity, **b** Reducing power and **(c)** DPPH radical scavenging activity of dried date (500 ppm concentration) Each observation is a mean ± SD of 3 replications In each figures means with same superscripts had no significant difference with each other ($P>0.05$)

contained the highest amount of phenolic compounds and also exhibited the strongest antioxidant capacity in all the assays used. Result of drying process showed that total phenolic content (TPC) and antioxidant activities (AA) varied with temperature and decreased by increase of drying temperature (from 667.3 to 610.5 mg galic acid in sun dried dates of Mozafati and Kaluteh respectively to 314.2 and 210.4 in dried dates (80 °C) of Mozafati and Kaluteh respectively).

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