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FRUIT DEVELOPMENTAL STAGES EFFECTS ON BIOCHEMICAL ATTRIBUTES IN DATE PALM

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Some date palm cultivars grown in Pakistan were biochemically characterized and the effect of fruit maturity on radical scavenging capacity (DPPH), total phenolic contents (TPC), specific activity of antioxidant enzymes, sugars profile and soluble protein contents was assessed. Higher range of differences in composition of studied phytochemicals was recorded among different cultivars. Antiradical efficiency (AE), TPC, antioxidant enzymes and soluble protein contents were recorded higher at khalal stage thereafter, declined at rutab then finally at tamar stage. The amount of glucose (11.32-32.50%) and fructose (10.95-32.41%) started accumulation from khalal stage and were in higher composition at tamar stage due to hydrolysis and inversion of sucrose (10.82-3.1%) contents. The results concluded that variation in biochemical attributes primarily influenced by type of cultivars and different fruit developmental stages.
Keywords: Dates, fruit maturity, antioxidants, sugars, total phenolic contents

INTRODUCTION

Date palm is a diploid (2n=36), perennial, monocotyledonous and diecious plant in family Arecaceae which comprises of 183 genera and 2600 species (Dowson, 1982; Dransfield *et al*., 2008). *Phoenix* is one of the genera which consist of 14 species, all native to the tropical and subtropical regions of Southern Asia or East and North Africa (Shengji *et al*., 2010). It is leading fruit tree in many countries and is an important source of food, sugar, nutrients and antioxidants. Currently, it is cultivated in the Middle East, North Africa, parts of Central and South America, Southern Europe, India and Pakistan (Chandra *et al*., 1992; Zaid, 1999; Al-Shahib and Marshall, 2003). Pakistan is the 6th largest producer of dates after Egypt, Saudi Arabia, Iran, Algeria and Iraq, with a production of 557279 tons (FAO, 2011). Date palm growth is affected by different factors (Afzal *et al*., 2011; Ata *et al*., 2012). Important dates producing provinces are Sindh (Khairpur and Sukkur), Balochistan (Makran and Panjgoor), Khyber Pakhtunkhwa (D. I. Khan) and Punjab at Jhang, Muzafargarh, Bahawalpur and D. G. Khan (Markhand *et al*., 2010).

Dates fruit is berry and oblong with fleshy mesocarp and fibrous endocarp (Mansour, 2005) which constitute 85-90% of total date fruit weight (Hussein *et al*., 1998). There are five developmental stages in fruits after pollination based on change in color, texture, aroma and flavor. Internationally accepted date fruit developmental stages are in Arabic terms, viz. Hababouk (immature and pea size), kimri (green, hard, contains 80% moisture and 50% reducing sugars (glucose and fructose), khalal (colored stage, crunchy, moisture contents upto 50-60%), rutab (ripe stage, soft texture, crisp to succulent, moisture content is 35-40%) and tamar (full ripe, dry flesh, moisture content less than 20%) (Al-Shahib and Marshall, 2003; Fadel *et al*., 2006). Dates can be harvested at khalal stage depending on sugar content, i.e. sweetness, climatic conditions and market demand. Date palm is affected by X-irradiation (Al-Enezi *et al*., 2012). Fresh dates are nutritionally superior and delicious than dried dates (Vinson *et al*., 2005; Al-Farsi *et al*., 2005).

Date fruits possessed a variety of enzymatic and non-enzymatic antioxidant compounds with varying amount in different cultivars (Mansouri *et al*., 2005; Biglari *et al*., 2008; Awad *et al*., 2011a). The significance of antioxidants has been increasing because of their high capability in scavenging free radicals associated to variety of many damaging diseases (Silva *et al*., 2007). Chemically, dates are considered rich source of sugars (mainly reducing) and protein contents (Amira *et al*., 2011; Rastegar *et al*., 2102) as compared to other fruits. The chemical composition of date palm varies within cultivars at different levels of maturity. It is therefore, ten date palm cultivars were selected to investigate their biochemical composition with an objective to characterize the superior cultivars with specific nutritional value at different developmental stage.

MATERIALS AND METHODS

***Plant material*:** The fruits of ten cultivars, i.e. Zerin, Jaman, Pela Dora, Rachna, Seib, Zardo, Shado, Peli Sunder, Wahan

Wali and Champa Kali were selected at three different developmental stages (khalal, rutab and tamar) during 2012 harvesting season from the Date palm Research Station, Jhang, Pakistan.

***Extraction of date flesh*:** Flesh of fruits (0.5g) of each cultivar was taken at each khalal, rutab and tamar stage and grinded in mortar by pestle with 2 ml methanol solution (95% v/v) at room temperature (25ºC±4) as described by Ainsworth and Gillespie (2007) to measure antioxidant activity and total phenolic contents. The extraction of date flesh in potassium phosphate buffer (pH: 7.0) was carried out as described by Naqvi *et al*. (2011) for estimation of specific activity of enzymes (catalase and peroxidase) and soluble protein contents. The extracts were filtered and centrifuged at 13,000x*g*, at 4ºC for 5 minutes. The residues were discarded and the supernatant were separated and stored at 4ºC until use.

***Radical scavenging assay (DPPH)*:** The antioxidant activity of the flesh of date fruits was assessed by measuring their scavenging abilities to 2, 2-diphenyl-1-picrylhydrazyl stable radicals as described by Amira *et al*. (2012). The absorbance was read against a blank at 517 nm using micro-plate ELISA reader (BioTek, USA). Inhibition of free radical by DPPH in percent (%) was calculated by following formula:

$$I\ \% = (A_{blank} - A_{sample} / A_{blank}) \times 100$$

Where A_{blank} is the absorbance of the control reaction mixture excluding fruit sample, and A_{sample} is the absorbance of the test compounds. IC_{50} values, which represented the concentration of date fruit extracts that caused 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

***Total phenolic contents (TPC)*:** TPC was calculated by using Folin-Ciocalteu reagent method as reported by Ainsworth and Gillespie, (2007). The FC-reagent (10 mL) was dissolved in distilled water to make the solution 100 mL. In each sample (100 mL), FC-reagent (200 µL) was added and vortex thoroughly. The 700 mM Na_2CO_3 (800 µL) was added into each sample and incubated at room temperature for 2 h. Sample (200 µL) was transferred to a clear 96-well plate and absorbance of each well was measured at 765 nm. Amount of TPC was calculated using a calibration curve for Gallic acid. The results were expressed as Gallic acid equivalent (GAE) per dry matter.

***Enzymatic antioxidant activity*:** The specific activity of antioxidant enzymes (catalase and peroxidase) were quantified using the method of Naqvi *et al*. (2011). Changes in absorbance of the reaction solution of catalase (CAT) and peroxidase (POD) were read at 240 nm and 470 nm, respectively.

***Sugars profiling (HPLC)*:** Different Sugar levels were measured using high-performance liquid chromatography (HPLC) technique as described by Amira *et al*. (2011). Sugars were extracted from date flesh (10g) in distilled water (20 mL) for 10 min (exciting repeatedly through magnetic stirrer in assistance dissolving the sugars). The extracts were then centrifuged at 13000x*g* for 10 min and the supernatants were separated. Each sample was filtered over 0.45 µm membrane filters and analyzed by high performance liquid chromatography (HPLC).

***Liquid chromatography conditions*:** LC separation was carried out at room temperature on a Razex RCM-Monosaccharides Ca^{+2}, Phenomenex. The mobile phase was 100% double distilled water. The HPLC was connected to a refractive index detector (R_eID) RID-10 AL (Shimadzu, Japan). The flow rate and injection volume were 1 mL/min and 20 µL, respectively. Identified sugars were quantified on the basis of peak areas of external standards consisting of glucose (1%), fructose (1%) and sucrose (1%) solutions. Total reducing sugars were obtained as a sum of glucose and fructose values. Each sample was carried out from integrated peak areas of the sample against the corresponding standard graph. Results were expressed as percentage of dry weight.

***Soluble protein contents*:** The soluble proteins of the fruit extracts were determined by Bradford method (Bradford, 1976) and absorbance was taken at 595 nm.

***Statistical analysis*:** The experimental values were analyzed statistically using one-way analysis of variance (ANOVA) with three replications of each treatment. Means were compared using Duncan's Multiple Range (DMR) test at 5% probability with the help of MINITAB (15.0).

RESULTS AND DISCUSSION

***Antiradical efficiency (DPPH)*:** There was a significant difference between antiradical efficiency (AE) of all selected date palm cultivars through the different developmental stages (Fig.1). Rachna cultivar showed the highest antiradical efficiency (3.63, 2.39 and 1.91 AE), followed by cv. Zardo cultivar (3.60, 2.56 and 1.17 AE at khalal, rutab and tamar stages, respectively. The cv. Wahan Wali showed lower (1.72, 1.33 and 1.21 AE) values at khalal, rutab and tamar stage, respectively. Selected date cultivars showed declining trend and depicted that AE was higher at khalal then gradually decreases at rutab and lower value was observed at tamar stage. The values and trend of current study was similar to those found by Awad *et al*. (2011a). Some reports referred that AE of date fruits was higher at khalal stage (Allaith, 2008; Amoros *et al*., 2009) which support the obtained results. Saafi *et al*. (2009) evaluated some Tunisian ripe fruits and found similar AE to those found in this study. The agro-climatic conditions may responsible for the found variation in the final values of the current study.

***Total phenolic concentration (TPC)*:** Total phenolic contents (TPC) of studied date fruits showed significant ($p < 0.05$) variation in the final values within cultivars and between stages of development as presented in Fig.2. Highest TPC was recorded in Jaman cultivar

(569.63<281.53<183.97 mg GAE/100g, DW), followed by cv. Rachna (508.91<265.58<159.84 mg GAE/100g, DW) and lowest values (348.9<169.88<59.73 mg GAE/100g, DW) was recorded in cv. Peli Sunder at khalal < rutab < tamar stage, respectively. It is often described that the values of TPC decreases from khalal to tamar stage and showed positive correlation with antioxidant activity of the date fruits in relation to maturity (Al-Turki *et al*., 2010; Awad *et al*., 2011a). This decrease in TPC is due to the oxidation of TPC by polyphenol oxidase (Amiot *et al*., 1995) and decline in tannins as the dates matured to the tamar stage (Myhara *et al*., 1999). Pakistani dates showed similar values to date cultivars from America and Saudi Arabia (Al-Turki *et al*., 2010) and Tunisia (Amira *et al*., 2012).

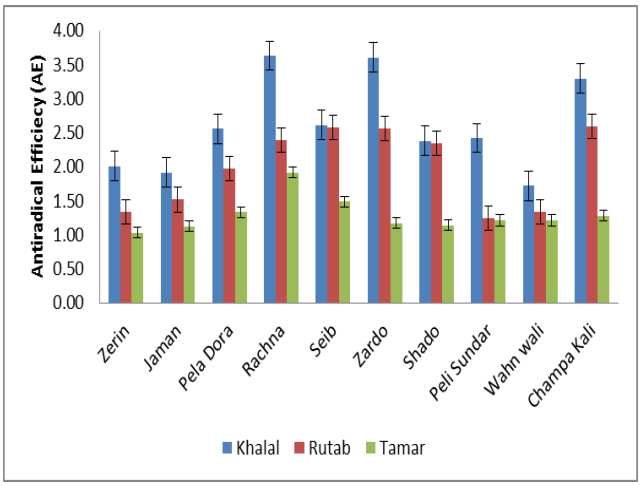

Figure 1. Antiradical efficiecy of ten date varities at three developmental stages

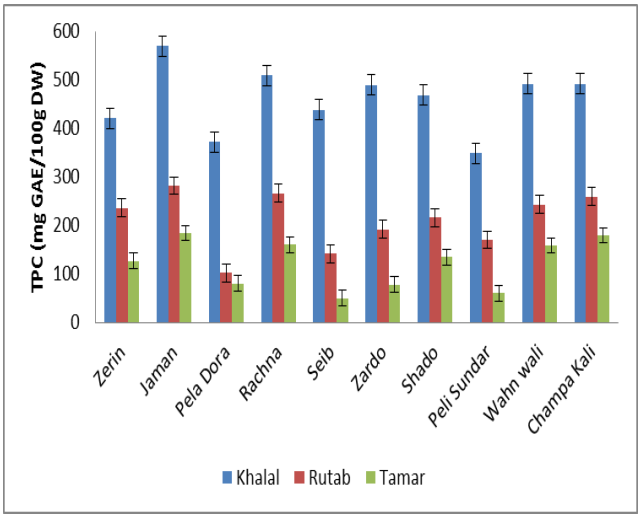

Figure 2. Total phenolic contents of ten date varities at three developmental stages

***Specific activity of antioxidant enzymes*:** The specific activity of antioxidant enzymes (catalase and peroxidase) was initially higher at khalal stage then sharply decreased during rutab and finally lowest at tamar stage in all cultivars. The specific activity of catalase (CAT) was higher at khalal in cvs. Peli Sunder and Zardo, afterwards sharply decreased at rutab in cvs. Seib and Wahan Wali cultivars then absolute decrease was observed in cultivars Zerin and Seib at tamar stage (Fig.3). Similarly, specific activity of peroxidase (POD) was higher at khalal in cvs. Wahan wali and Champa Kali culivars then gradually decrease at rutab in cvs. Champa Kali and Rachna. Final decrease of Specific POD activity was recorded in cvs. Zardo and Peli Sunder at fully ripened (tamar) stage (Fig.4). Awad *et al*. (2011a) studied antioxidant enzymes (CAT and POD) and depicted that their activity was higher at khalal stage and thereafter, declined at rutab and tamar stage, respectively and was in good agreement to those found in our studied cultivars.

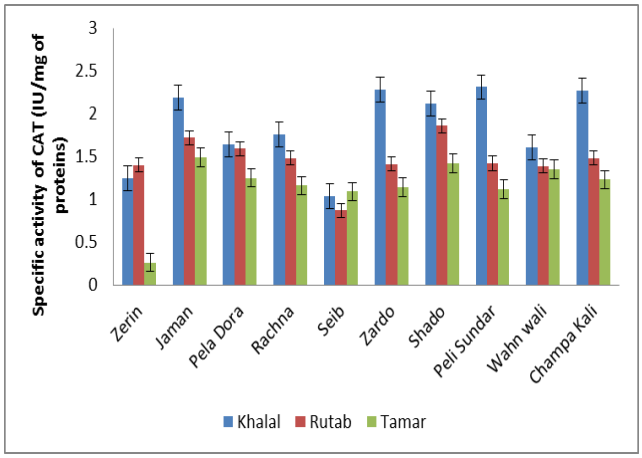

Figure 3. Specific activity of catalase of ten date varities at three developmental stages

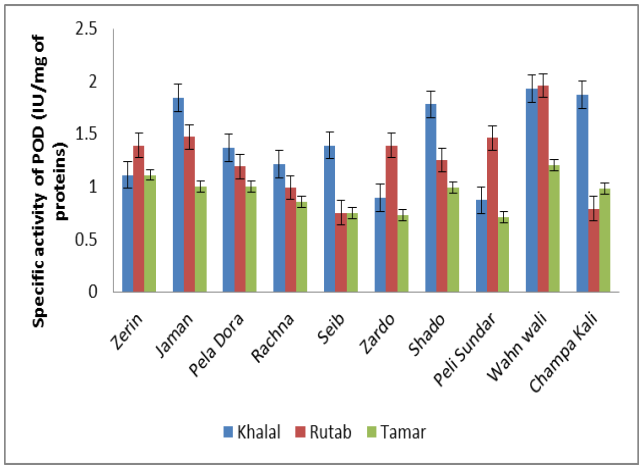

Figure 4. Specific activity of peroxidase of ten date varities at three developmental stages

Table 1. Sugar profile of ten date palm cultivars at three different developmental stages as quantified by HPLC method

Cultivars	Ripening stage	Sucrose (%)	Glucose (%)	Fructose (%)	Reducing Sugars (%	G/F2
Zerin	Khalal	10.82±0.6j	16.65±1.1h	14.47±0.5h	31.12±0.2h	1.15
	Rutab	5.76±0.2d	20.81±0.4g	20.25±1f	41.04±0.9f	1.02
	Tamar	nd1	25.52±0.87f	24.91±0.3f	50.43±0.2f	1.02
Jaman	Khalal	15.59±1.2c	20.32±1.13b	18.32±0.8c	38.64±1.1c	1.10
	Rutab	4.7±0.8h	24.57±0.3c	24.02±0.4b	48.58±0.3b	1.02
	Tamar	nd	30.06±0.4b	29.65±0.7b	59.7±0.7b	1.01
Pela Dora	Khalal	12.82±0.5h	17.00±1.06g	15.56±1.1g	32.59±0.9g	1.09
	Rutab	6.2±0.7c	20.50±0.5h	19.66±0.9g	40.16±0.7g	1.04
	Tamar	nd	24.91±0.9h	23.58±0.7h	48.49±0.2h	1.06
Rachna	Khalal	13.79±0.3g	13.10±0.99i	11.55±0.03i	24.65±0.8i	1.13
	Rutab	4.8±0.01g	16.55±1i	15.90±0.2i	32.45±0.3i	1.04
	Tamar	nd	21.95±0.7i	19.94±0.4i	41.89±1.01i	1.10
Seib	Khalal	19.89±0.7a	21.79±0.9a	20.45±0.5a	42.24±0.9a	1.06
	Rutab	5.21±0.2e	28.74±1.1a	26.4±1.3a	55.14±0.5a	1.09
	Tamar	nd	32.50±1.02a	32.41±1a	64.91±0.03a	1.00
Zardo	Khalal	15.71±1.2b	17.65±1.1f	17.21±0.9d	34.86±1e	1.03
	Rutab	3.1±1j	23.54±0.7d	22.61±0.7c	46.15±0.7d	1.04
	Tamar	nd	27.33±0.98e	26.81±1.2c	54.14±0.4d	1.01
Shado	Khalal	15.16±0.4d	20.23±0.8c	19.1±0.6b	39.33±1.3b	1.05
	Rutab	6.4±0.3b	24.82±0.3b	21.69±0.3d	46.51±1.2c	1.14
	Tamar	nd	29.30±0.2c	26.6±1.01d	55.9±0.6bc	1.10
Peli Sunder	Khalal	14.55±0.02f	11.32±1.02j	10.95±1.3j	22.26±1.3j	1.03
	Rutab	4.32±1.1i	15.83±0.6j	14.76±1j	30.58±0.7j	1.07
	Tamar	nd	20.1±0.9j	18.21±0.3i	38.31±1j	1.10
Wahan Wali	Khalal	14.66±0.9e	17.89±1.03e	16.8±0.7e	34.67±0.9f	1.06
	Rutab	5.31±0.3f	21.34±1e	20.48±0.7e	41.82±0.7e	1.04
	Tamar	nd	27.65±0.2d	25.93±0.8e	53.59±0.3e	1.06
Champa Kali	Khalal	12.26±0.1i	18.74±1.2d	16.97±1f	35.7±1.1d	1.10
	Rutab	7.32±0.2a	20.95±0.2f	18.35±0.5h	39.29±0.2h	1.10
	Tamar	nd	25.16±1.1g	23.76±0.2g	48.91±0.9g	1.06

***Estimation of sugars (HPLC)*:** The amount and composition of most important sugars are shown in Table 1. Analysis of variance revealed significant ($p<0.05$) variations between the values of sucrose and reducing sugars (glucose and fructose) among all selected date cultivars and within maturation stages. The quantity and type of sugars vary within cultivars and at stages of maturity. The amount of reducing sugars ranged (22.26-42.24 %), (30.58-55.14 %) and (38.31-64.91 %) at khalal, rutab and tamar, respectively; whereas, sucrose contents ranged (12.82-19.9 %) at khalal stage and some minor quantity were also detected at rutab stage. The cv. Seib possessed higher total sugar contents but cv. Peli Sunder showed lower values at all edible stages of date fruit development. The sucrose contents were not detected at tamar stage due to hydrolysis and later conversion into reducing sugars by the invertase enzyme (Al-Farsi and Lee,

2008). Amira *et al*. (2011) quantified the sugars from Tunisian date cultivars and reported similar findings of reducing sugars at all developmental stages. Values of reducing sugars in our results were similar to those cultivars reported by (Vayalil, 2011; Rastegar *et al*., 2012).

***Quantification of soluble protein contents*:** The soluble protein contents of date fruits are expressed in g/100 g (Fig.5). The soluble protein contents were found higher at khalal stage and substantially declined at rutab then tamar stage, respectively. The soluble protein contents ranged (5.22-5.68 g/100g), (3.67-4.56 g/100g) and (2.87-3.42 g/100g) at khalal, rutab and tamar stages respectively. The Zardo and Rachna cultivars shower higher protein contents at khalal, the cvs. Wahan Wali and Jaman at rutab and cvs. Zardo and Peli Sunder at tamar stage. This declining trend of soluble proteins may be due to its degradation by free radicals as the radical scavenging system declines during

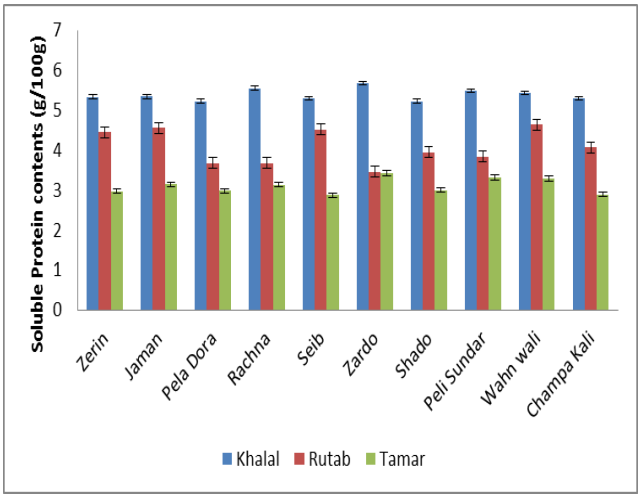

Figure 5. Soluble protein contents of ten date cultivars at three developmental stages

maturity (Prochazkova *et al*., 2001) and the rising activities enzymes like proteases (Rastegar *et al*., 2012). The soluble protein contents in this study decreases from kimri to tamar stage of date fruit maturity that is in good agreement to the date cultivars reported by Awad *et al*. (2011b).

***Conclusion*:** This study revealed that exotic date palm germplasm available in Pakistan are the potential source of radical scavenging capability, comparable reducing sugars and protein contents compared to other famous local date palm cultivars like Aseel and Dhakki. These cultivars can be brought under consideration for cultivation as commercial varieties in future.

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