Pak. J. Bot., 50(3): 1069-1076, 2018.

POMOLOGICAL AND BIOCHEMICAL PROFILING OF DATE FRUITS (*PHOENIX DACTYLIFERA* L.) DURING DIFFERENT FRUIT MATURATION PHASES

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Abstract

Date palm (*Phoenix dactylifera* L.) is an important part of human diet due to the impressive panel of sugars, polyphenols, antioxidants and essential elements. In this study, ten elite indigenous Pakistani date palm cultivars were characterized for their fruit morphological and biochemical traits at three fruit ripening stages. Results depicted significant distinction in size, shape and fruit dimensions during their different softening patterns. Moreover, the moisture contents, soluble protein contents, total phenolic contents (TPC), antioxidant activity (AA), specific activity of catalase (CAT) and peroxidase (POD) were high at khalal stage, followed by rutab stage, and lowest at tamar stage. On the converse, pH and reducing sugars (glucose and fructose) increased up to the full-ripe stage in all the investigated cultivars. Pearson's test was also established in fruit morphological parameters and sugar components. The disparity in nutritional composition mainly depended on the type of cultivar and fruit maturation stages. Our findings revealed that the indigenous date palm germplasm was the potential source of sugar contents and variety of antioxidants and could possibly be used as functional food components.

Key words: Date palm, Soluble sugars, Morphology, Nutrition, HPLC.

Introduction

Date palm, with the long history of cultivation, is regarded as an important fruit crop for the people living in the desert and hot regions of the world (Abdolvand *et al.*, 2018). This fruit plant is undeviatingly associated with the well-being of the farmers of particular realms (Aljasass *et al.*, 2016; Sami *et al.*, 2016). Free radicals, such as peroxyl radical (ROO.), superoxide anion ($O_2.^-$) and hydroxyl radicals (OH) are the unpaired electrons that have a significant role in the pathogenesis of cancer, diabetes, inflammation, cardiovascular, neurodegenerative disorder, atherosclerosis as well as in other pernicious infections (Baliga *et al.*, 2011). Various complexes, such as antioxidant compounds (phenolics, flavonoids, soluble tannins and ascorbic acid), carotenoids and tocopherols can act as a single oxygen, and lipid peroxidation quenchers, consequently possessing the capacity to counterbalance the free radicals implicated in the oxidative progressions through conjoining with oxidizing species or hydrogenation, and help in depreciating the disease risk (Al-Farsi *et al.*, 2005, Amira *et al.*, 2012, Haider *et al.*, 2013).

Date fruits have the important scavenging ability that can quench the free radical due to the presence of antioxidant activity, antioxidative compounds (total phenolic contents, total flavonoid, and ascorbic acid) and enzymatic antioxidative activity (SOD, POD and CAT) (Awad *et al.*, 2011; Amira *et al.*, 2012). Moreover, dates are also considered the prompt source of sugars (mainly glucose and fructose) and protein compared to other fruits worldwide. Various factor, including cultivar, genome, climatic conditions, irrigation, sunlight and post-harvest treatments may affect the radical scavenging activity, sugar and protein compositions of fruits (Awad *et al.*, 2011, Baliga *et al.*, 2011; Alhamdan *et al.*, 2016). In addition, developmental stages are also responsible for influencing the compositional quality of fruits and vegetables, as many physiological, biochemical and structural variations occur during maturation process that ensures fruit quality parameters (Haider *et al.*, 2013). Fruit harvesting at the mature stage is very important for better quality but mainly depends on climatic conditions, cultivar type and market demand (Al-Farsi *et al.*, 2007).

Date palm (*Phoenix dactylifea* L.) is the 4th important fruit crop after mango, citrus and banana, which plays a beneficial role in the social and economic welfare of the population, living in the hot and dry arid region of Pakistan (Maryam *et al.*, 2015; Mirbahar *et al.*, 2016). Date palm industry is well established in Pakistan and ranked 5th position in terms of production, sharing 10.2% in the total world production (Haider *et al.*, 2015; Naqvi *et al.*, 2015). There exist more than 300 cultivars along with various indigenous cultivars, being cultivated in all provinces for food and feed. Local date palm germplasm, including Aseel, Dhakki, Begum Jungi are the potential source of various nutritional components (Haider *et al.*, 2013; Haider *et al.*, 2104). Generally, there are four internationally accepted date fruit maturation stages, viz Kimri (green, firm, high tannins), Khalal (firm, crunch, colored), Rutab (half-ripe) and Tamar (ripe, soft texture) (Baliga *et al.*, 2011). However, the dates are picked at the khalal stage to make Chohara (dried-date) in Punjab and Sindh provinces of Pakistan (Haider *et al.*, 2013), but

overall consumed at rutab and tamar stages depending on the cultivar characters, sugar contents, soluble tannins, and market demand (Al-Qurashi, 2010). The phytochemical composition of date fruits like antioxidant activity and phenolic contents vary during progressive developmental stages and may affect the shelf life (Al-Farsi *et al.*, 2005, Haider *et al.*, 2014). The selected indigenous Pakistani date palm cultivars have not yet been evaluated for their fruit morphological and nutritional characterization. Therefore, in this study, ten different cultivars were evaluated for their various morphological and nutritional attributes at their three-different progressive developmental stages (khalal, rutab, and tamar). Results will set the foundation to understand the nutritional composition with respect to different edible stages of date palm cultivars.

Materials and Methods

Ten date palm cultivars, including Zehdi, Be-Rehmi, Neelum, Ko-Herba, Kozan Abad, Karblian, Jan-Sohar, Khadrawy I, Khadrawy II, Angoor were selected from the Date Palm Research Station, Jhang-Pakistan. The fruits at three different developmental stages *i.e.*, khalal, rutab and tamar were harvested. Selected fruits were characterized for morphological and biochemical analysis, whose details are given below.

Morphological analysis: Fifteen fruits from each cultivar were selected for morphological analysis, like fruit dimensions (length and width), seed dimensions (length and width), fruit/pulp ratio, weight (fruit and pulp). The dimension of fruit and seed were first recorded by using the micrometer caliper (Guido *et al.*, 2011), fruit weight was taken by the digital weight balance and fruit to pulp ratio was also calculated.

Extraction of date flesh: The edible part (pulp) of date palm fruits (0.5 g) at three maturity stages was ground using 2 mL of methanol (95% V/V) by following the method of Ainsworth & Gillespie (2007). Extraction in distilled water and phosphate buffer (pH = 7.0) was carried out in the same manner for estimation of sugars and enzymes, respectively. These extractions were carried out in standard (25 °C ± 4) room conditions.

Proximate composition analysis

Moisture contents: For moisture contents estimation, 3 g of date pulp was placed on the stainless capsule and then oven dried at 80°C until constant weight obtained. The final results are shown as a percentage on a fresh weight basis.

pH: The pH of date extract was measured using pH meter following the method of Guido *et al.*, (2011).

Soluble protein content: For protein contents estimation, Bradford reagent (2 mL) was mixed in 50 µL of date fruit extract and the absorbance was taken at 595 nm using the blank contains Bradford reagent, following the method of Bradford, (1976). Bovine serum albumin (BSA) standard curve was used for the protein contents quantification.

Identification and quantification of sugars using HPLC: High-performance liquid chromatography (HPLC) technique was used for estimation and quantification of sugars as reported by Guido *et al.*, (2011). The separation was carried out at room temperature on a Razex RCM-Monosaccharides Ca, phenomenex. The mobile was 100% (v/v) double distilled water. The HPLC was connected to a refractive index detector (R_e ID) RID-10AL (Shimadzu, Japan). The injection volume and flow rate was 20 µL and 0.6mL/min, respectively. Identification sugars were quantified on the bases of the peak areas of external standards consisting of glucose (1%), fructose (1%) and sucrose (1%) solutions. Each sample was carried out from integrated peak areas of the sample against the corresponding standard graph. Results were expressed as a percentage of dry weight.

Total phenolic content (TPC): Folin- Ciocalteu (FC)-reagent method was used for the TPC determination as already described by Ainsworth & Gillespie, (2007). 200 µL of FC-reagent was added in the 100 mL of date extracts and then 800 µL of 700mM Na_3CO_3 was added into each sample, and vortexed thoroughly. After 2 h incubation at room temperature, the mixture (200 µL) was centrifuged in 96 well plate with three replicates, and reading was taken at 765 nm. The Gallic acid (GA) standard curve was used for the quantification of TPC. The results were expressed as Gallic acid equivalent (GAE).

Antiradical efficiency by DPPH-assay: The radical scavenging ability of the date palm fruits was measured by using the 1, 1-diphenyl-1-picrylhydrazyl (DPPH) stable radicals against a blank reading at 517 nm, as described by Haider *et al.*, (2013). The inhibition percentage of the free radicals were calculated by the 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 cause 50% neutralization of DPPH radicals, were calculated from the plot of inhibition percentage against concentration.

Enzymatic antioxidant activity: The specific activities of catalase (CAT) and peroxidase (POD) was measured at 240 nm and 470 nm, respectively using the method of Naqvi *et al.*, (2011). Hydrogen peroxide is being used as a substrate by the CAT; whereas, POD generates water and activated donor molecule.

Statistical analysis: Completely Randomized Design (CRD) was used to analyze one-way analysis of variance (ANOVA) to record significant difference of studied parameters and mean values were compared at least significant difference using Duncan's Multiple Range (DMR) at ($p = 0.05$) using IBM SPSS 20.0 (SPSS Inc, Chicago, IL, U.S).

Results

Morphological characterization of date fruits: There existed significant ($p<0.05$) differences among cultivars in their fruit morphology according to their different maturation stages (Table 1). These differences were very prominent for the pulp and fruit weight. A significant decrease in fruit and pulp weight (5.01 ± 0.67 - 13.40 ± 0.72 g) and (4.18 ± 0.98 – 12.33 ± 0.85 g), respectively, from khalal to tamar stage, was recorded in selected date palm varieties. Moreover, dimensions of date fruits were also decreased from 1.73 ± 0.57 to 1.20 ± 0.53 cm for length, and 0.63 ± 0.03 to 0.38 ± 0.02 for width. During the fruit maturation phase, Cvs. Be-Rehmi and Jan-Sohar had highest fruit length (1.73 ± 0.42 – 1.65 ± 0.48 and 1.73 ± 0.57 – 1.26 ± 0.51 cm) and width (0.61 ± 0.17 – 0.47 ± 0.07 and 0.55 ± 0.05 – 0.54 ± 0.04 cm), respectively. Contrarily, cv. Angoor had smallest value of length (1.46 ± 0.57 - 1.20 ± 0.53 cm) and width (0.50 ± 0.03 - 0.38±0.02 cm) until ripened (tamar) stage. The differences in dimensions of date fruit are may be due to cultivar type, genetics, and climatic conditions.

Proximate analysis composition: The moisture contents of date fruits were negatively correlated with the sugars contents. There existed significant ($p<0.05$) differences between cultivars and fruit maturation stages. The moisture content percentage ranged from 70.2 ± 0.38% in cv. Ko-Harba at the khalal stage to 14.86 ± 0.21% in cv. Angoor at tamar stage. Zehdi showed a drastic decrease of moisture contents (54.34 ± 0.9 - 29.77 ± 0.86%) from khalal to rutab stage then afterward little decrease was observed at tamar stage. On the contrary, an increase was observed in pH value ranging between 5.91 ± 0.3 and 6.78 ± 0.2 during date fruit development phase (Table 2). The final value of soluble protein contents was higher at khalal stage and then decreased at tamar stage, also revealed significant ($p<0.05$) differences among ten selected date palm cultivars (Table 3). The maximum level of soluble protein contents expressed as (g/100g FW) was recorded in cv. Karblain (5.75 < 4.78 < 3.46 g/100 g FW), followed by cv. Jan-Sohar (5.61 < 4.57 < 3.07 g/100 g FW) at khalal < rutab < tamar, respectively.

Identification and quantification of sugars: The important sugars' profile and quantity in date palm cultivars during their maturation as quantified using HPLC method are presented in Table 2. There existed significant differences ($p < 0.05$) between the final values of reducing (glucose and fructose) and non-reducing (sucrose) sugars of all examined date cultivars. The sugar type and its quantity showed variation between different cultivars, and even within different maturation phases of fruits. The reducing sugars composition increased significantly ($p<0.05$) with respect to fruit developmental stages, from 21.07 ± 0.6% in cv. Kozan Abad at the khalal stage to 59.36 ± 0.9% (DW) in Angoor at tamar stage. Angoor (41.74 ± 0.3-59.36 ± 0.9% DW) and Zehdi (41.21 ± 0.2-59.03 ± 0.2% DW) cultivars showed higher reducing sugars level, while lowest quantity was recorded in cv. Kozan Abad (21.07 ± 0.6-41.80 ± 1.2% DW) from khalal to tamar, respectively. On the contrary, the sucrose contents showed decreasing activity during ripening process and were not detected in all cultivars at tamar stage except some cultivars showed minor quantity at tamar stage including, Khadrawy-I (16.39 ± 1.1-3.63 ± 0.2% DW), Zehdi (17.02 ± 0.6-2.63 ± 0.2%), Ko-Harba (18.11 ± 1.1-2.31 ± 0.4% DW) and Khadrawy-II (15.15 ± 0.9-1.75 ± 0.2% DW).

Total phenolic content (TPC): The composition of TPC of date palm cultivars during maturation process has been represented in table 3. The data regarding TPC have indicated the significant differences ($p<0.05$) between cultivars and within different fruit developmental stages. The TPC was observed higher at khalal stage (354.54 ± 1.19 mg GAE/100 g DW) in cv. Ko-Herba, gradually decrease at the rutab stage, then afterward finally reached the lowest level at tamar stage (26.93 ± 0.23 mg GAE/100 g DW). In general, cv. Karblain exhibited overall optimum composition (339.39 ± 1.37-151.63 ± 1.54 mg GAE/100 g DW); whereas, Jan-Sohar possessed lowest (117.09 ± 1.48-107.63 ± 0.86 mg GAE/100 g DW) TPC during maturation ranging khalal to tamar stages, respectively.

Antioxidant activity by DPPH-Assay: Antioxidants have the ability to counteract the damaging effects of oxidation caused by free radicals. Date fruit also has the antioxidant activity (AA). In this experiment, the AA expressed antiradical efficiency (AE = $1/IC_{50}$) unveiled significant differences ($p<0.05$) in all examined date palm cultivars during fruit developmental process (Table 3). The AA also followed the similar trend with TPC and gradually decreased from khalal to tamar respectively. Cv. Kozan Abad showed maximum level of AA (3.25, 2.96, 2.13 AE), followed by cv. Jan-Sohar (3.10, 2.61, 1.39 AE) at khalal, rutab, and tamar, respectively. Whereas, cv. Karblain showed the lowest AA (1.69-1.27 AE) from khalal to tamar stage, respectively.

Specific enzymatic antioxidant activity: The specific activity of antioxidant enzymes, including catalases (CAT) and peroxidases (POD), presented significant differences ($p<0.05$) among fruits of different date cultivars during ripening stages. The specific activity of CAT was higher in cv. Karblain (2.97 ± 0.12-1.08 ± 0.24 13 IU/mg of protein), followed by cv. Neelum (2.70 ± 0.34-0.57 ± 0.31 13 IU/mg of protein) and lowest activity was recorded in Kozan Abad (1.48 ± 0.6-1.33 ± 0.15 IU/mg of protein) from khalal to tamar stage, respectively (Table 3). Moreover, the specific activity of POD followed the same trend and was detected higher at khalal in cvs. Karblain and Ko-Harba (3.79 ± 0.66 and 2.86 ± 0.19 IU/mg of protein), then decreased at a rutab stage in cvs. Karblain and Angoor (2.95 ± 0.49 and 2.81 ± 0.23 IU/mg of protein) and the absolute decrease was recorded in cvs. Karblain and Khadrawy-II (1.79 ± 0.13 and 1.78 ± 0.12 IU/mg of protein), respectively.

Table 1. Morphological attributes of fresh date fruits of ten different cultivars according to their progressive maturation phases

Samples	Ripening stage	Fruit dimensions (cm)		Seed dimensions (cm)		Weight (g)		Pulp/Fruit ratio (%)
		Length	Width	Length	Width	Fruit	Pulp	
Zehdi	Khalal	1.41 ± 0.55a	0.53 ± 0.02a	0.97 ± 0.27a	0.17 ± 0.01a	8.90 ± 0.77b	7.44 ± 1.05c	83.59 ± 1.15b
	Rutab	1.33 ± 0.43b	0.51 ± 0.01a	1.01 ± 0.25ab	0.18 ± 0.01ab	8.30 ± 0.82c	7.33 ± 0.97ab	88.31 ± 1.09a
	Tamar	1.25 ± 0.52c	0.45 ± 0.07bc	0.96 ± 0.25b	0.11 ± 0.02c	7.91 ± 0.72d	7.13 ± 0.95d	90.13 ± 1.13c
Be-Rehmi	Khalal	1.73 ± 0.42a	0.61 ± 0.17a	0.90 ± 0.22a	0.16 ± 0.01ab	9.20 ± 0.67b	8.24 ± 0.99c	89.60 ± 1.03a
	Rutab	2.00 ± 0.51a	0.59 ± 0.05a	0.95 ± 0.27abc	0.17 ± 0.02b	8.40 ± 0.75c	7.35 ± 1.03ab	87.34 ± 1.11a
	Tamar	1.65 ± 0.48a	0.47 ± 0.07b	0.92 ± 0.21c	0.17 ± 0.07b	7.71 ± 0.72e	6.84 ± 1.01e	88.71 ± 0.97d
Neelum	Khalal	1.50 ± 0.49a	0.55 ± 0.03a	0.99 ± 0.31a	0.16 ± 0.02ab	8.43 ± 0.75a	7.45 ± 0.97c	87.90 ± 1.13ab
	Rutab	1.35 ± 0.56b	0.55 ± 0.02a	1.03 ± 0.29a	0.18 ± 0.04ab	7.10 ± 0.76d	5.92 ± 0.87ab	83.16 ± 1.09a
	Tamar	1.32 ± 0.52bc	0.44 ± 0.03c	1.00 ± 0.27a	0.18 ± 0.01ab	6.71 ± 0.69f	5.22 ± 0.93i	77.79 ± 1.02i
Ko-Harba	Khalal	1.67 ± 0.57a	0.57 ± 0.01a	0.94 ± 0.23a	0.17 ± 0.02a	9.13 ± 0.82b	8.04 ± 0.99c	88.07 ± 1.02ab
	Rutab	1.63 ± 0.55ab	0.58 ± 0.04a	0.92 ± 0.2bc	0.18 ± 0.07ab	8.10 ± 0.73c	6.97 ± 0.89ab	85.88 ± 1.04a
	Tamar	1.36 ± 0.55bc	0.46 ± 0.05bc	0.87 ± 0.23e	0.18 ± 0.01ab	7.91 ± 0.73d	6.70 ± 0.87g	84.70 ± 1.03f
Kozan Abad	Khalal	1.48 ± 0.52a	0.48 ± 0.03a	0.99 ± 0.26a	0.12 ± 0.02b	9.70 ± 0.72b	8.65 ± 0.95bc	89.07 ± 1.07a
	Rutab	1.43 ± 0.53ab	0.58 ± 0.03a	0.91 ± 0.27bc	0.19 ± 0.02ab	8.50 ± 0.81c	7.29 ± 1.05ab	85.61 ± 1.05a
	Tamar	1.48 ± 0.58abc	0.46 ± 0.03bc	0.88 ± 0.27de	0.19 ± 0.04ab	7.71 ± 0.69e	6.79 ± 1.07f	88.06 ± 1.03e
Karblain	Khalal	1.61 ± 0.56a	0.60 ± 0.02a	0.89 ± 0.3a	0.17 ± 0.01a	9.60 ± 0.67b	8.58 ± 0.98bc	89.37 ± 1.05a
	Rutab	1.80 ± 0.53ab	0.58 ± 0.3a	0.94 ± 0.27abc	0.18 ± 0.03ab	9.20 ± 0.82b	8.03 ± 0.88ab	87.15 ± 0.98a
	Tamar	1.55 ± 0.53ab	0.46 ± 0.02bc	0.90 ± 0.25cd	0.18 ± 0.02ab	7.71 ± 0.74e	6.37 ± 0.87h	82.62 ± 1.19h
Jan-Sohar	Khalal	1.73 ± 0.57a	0.55 ± 0.05a	1.22 ± 0.26a	0.19 ± 0.07a	13.40 ± 0.72a	12.33 ± 0.85a	92.04 ± 1.01a
	Rutab	1.51 ± 0.56ab	0.67 ± 0.04a	0.85 ± 0.25cd	0.20 ± 0.02a	12.10 ± 0.77a	10.82 ± 0.93a	89.34 ± 1.03a
	Tamar	1.26 ± 0.51c	0.54 ± 0.04a	0.82 ± 0.29f	0.20 ± 0.04a	11.41 ± 0.75b	10.32 ± 0.93b	90.45 ± 1.08c
Khadrawy-I	Khalal	1.70 ± 0.54a	0.63 ± 0.03a	0.90 ± 0.22a	0.18 ± 0.01a	13.30 ± 0.75a	11.83 ± 0.95ab	88.96 ± 0.99a
	Rutab	1.83 ± 0.55ab	0.65 ± 0.03a	0.94 ± 0.23abc	0.17 ± 0.04b	11.90 ± 0.72a	10.23 ± 1.02a	85.95 ± 1.12a
	Tamar	1.57 ± 0.5ab	0.54 ± 0.04a	0.92 ± 0.21c	0.17 ± 0.02b	11.01 ± 0.68c	10.05 ± 1c	91.28 ± 1.1b
Khadrawy-II	Khalal	1.57 ± 0.53a	0.60 ± 0.02a	0.81 ± 0.23a	0.18 ± 0.03a	13.20 ± 0.79a	12.13 ± 0.96a	91.89 ± 1.02a
	Rutab	1.57 ± 0.52ab	0.66 ± 0.01a	0.86 ± 0.24cd	0.18 ± 0.01ab	12.10 ± 0.8a	10.85 ± 0.95a	89.60 ± 1.06a
	Tamar	1.32 ± 0.55bc	0.54 ± 0.01a	0.83 ± 0.23f	0.18 ± 0.07ab	11.51 ± 0.75a	10.64 ± 0.96a	92.44 ± 1.06a
Angoor	Khalal	1.46 ± 0.57a	0.50 ± 0.03a	0.81 ± 0.31a	0.18 ± 0.02a	8.43 ± 0.72a	7.46 ± 0.97c	87.62 ± 1.02ab
	Rutab	1.46 ± 0.54ab	0.49 ± 0.01a	0.79 ± 0.29d	0.18 ± 0.01ab	5.60 ± 0.72e	4.47 ± 0.91b	79.48 ± 1.04a
	Tamar	1.20 ± 0.53c	0.38 ± 0.02d	0.76 ± 0.22g	0.18 ± 0.02ab	5.01 ± 0.67g	4.18 ± 0.98j	83.43 ± 1.02g

Table2. Sugar and moisture content, and pH level of Pakistani date palm cultivars at different fruit developmental stages

Cultivars	Ripening stage	Sucrose (%)	Reducing sugar (%)	Glucose (%)	Fructose (%)	G/F	Moisture (%)	pH
Zehdi	Khalal	17.02 ± 0.6bc	41.21 ± 0.2a	22.51 ± 0.8a	18.70 ± 0.2d	1.20	54.34 ± 0.9i	5.43 ± 0.1f
	Rutab	8.30B0.2a	50.94 ± 0.9a	25.19 ± 0.98a	25.75 ± 0.5a	0.98	29.77 ± 0.86f	6.05 ± 0.2b
	Tamar	2.63 ± 0.2b	59.03 ± 0.2b	29.42 ± 1.02a	29.61 ± 1a	0.99	24.21 ± 0.8b	6.57 ± 0.1abc
Be-Rehmi	Khalal	12.84 ± 1.2e	28.09 ± 0.9f	13.45 ± 1.1d	14.64 ± 0.81e	0.92	61.22 ± 0.48e	5.75 ± 0.1bc
	Rutab	3.12 ± 0.8e	38.34 ± 1.1f	18.39 ± 0.92g	19.95 ± 0.76d	0.92	33.57 ± 0.59c	6.13 ± 0.2b
	Tamar	0	49.29 ± 0.2h	23.49 ± 0.73d	25.80 ± 0.7f	0.91	23.31 ± 0.51d	6.76 ± 0.1ab
Neelum	Khalal	14.71 ± 0.2d	39.10 ± 1.1b	19.36 ± 1b	19.74 ± 0.3bc	0.98	64.29 ± 0.8d	5.64 ± 0.3de
	Rutab	4.99 ± 0.1d	44.71 ± 0.3e	21.49 ± 0.99e	23.56 ± 0.66c	0.91	33.79 ± 0.76c	6.10 ± 0.2b
	Tamar	0	54.08 ± 0.7e	27.54 ± 0.9b	26.54 ± 1.3e	1.03	20.74 ± 0.7g	6.63 ± 0.2abc
Ko-Harba	Khalal	18.11 ± 1.1ab	24.15 ± 0.9g	11.36 ± 0.5e	12.79 ± 1.0f	0.88	70.2 ± 0.38a	5.91 ± 0.3a
	Rutab	8.39 ± 0.9ab	33.78 ± 1h	17.76 ± 1.06h	16.02 ± 0.3g	1.11	40.64 ± 0.49a	6.06 ± 0.3b
	Tamar	2.31 ± 0.4c	46.92 ± 0.7i	22.56 ± 0.43e	24.36 ± 0.6g	0.93	23.40 ± 0.41d	6.53 ± 0.2abc
Kozan Abad	Khalal	16.25 ± 0.3c	21.07 ± 0.6h	9.71 ± 0.30f	11.36 ± 1.2g	0.85	56.72 ± 0.67g	5.84 ± 0.2ab
	Rutab	6.53 ± 0.2c	32.66 ± 1.3i	15.63 ± 1.13i	17.03 ± 0.7f	0.91	29.73 ± 0.63f	6.09 ± 0.1b
	Tamar	0	41.80 ± 1.2j	19.92 ± 0.87f	21.88 ± 0.9h	0.91	16.60 ± 0.57h	6.78 ± 0.2a
Karblain	Khalal	14.95 ± 0.3d	37.32 ± 0.4c	17.74 ± 0.46c	19.25 ± 1.01cd	0.92	56.76 ± 0.25g	5.44 ± 0.1f
	Rutab	5.23 ± 0.4d	45.20d ± 0.7e	21.75 ± 0.17d	23.45 ± 1.3c	0.92	32.19 ± 0.36d	6.18 ± 0.3b
	Tamar	0	55.96 ± 0.03c	27.18 ± 0.13b	28.78 ± 0.5bc	0.94	26.63 ± 0.28a	6.52 ± 0.2abc
Jan-Sohar	Khalal	12.09 ± 0.2e	28.69 ± 0.5e	13.96 ± 0.02d	14.73 ± 0.4e	0.94	59.12 ± 0.73f	5.72 ± 0.3cd
	Rutab	2.37 ± 0.9e	37.80 ± 1g	19.74 ± 0.13f	18.06 ± 0.2e	1.09	31.48 ± 0.69e	6.15 ± 0.4b
	Tamar	0	50.41 ± 0.5g	23.76 ± 0.07d	26.65 ± 0.03e	0.89	21.22 ± 0.63f	6.71 ± 0.2abc
Khadrawy-I	Khalal	16.39 ± 1.1c	33.88 ± 0.3d	13.96 ± 0.58d	19.92 ± 0.7bc	0.70	67.40 ± 0.31c	5.62 ± 0.7e
	Rutab	7.67 ± 0.4bc	45.57 ± 0.8d	22.07 ± 0.12c	23.50 ± 0.9c	0.93	36.90 ± 0.42b	6.35 ± 0.3a
	Tamar	3.63 ± 0.2a	53.68 ± 0.2f	26.14 ± 0.13c	27.54 ± 1.1d	0.95	23.85 ± 0.34c	6.60 ± 0.3abc
Khadrawy-II	Khalal	15.15 ± 0.9d	39.33 ± 0.7b	19.10 ± 0.22b	20.23 ± 0.7b	0.94	69.21 ± 0.63b	5.86 ± 0.4a
	Rutab	6.43 ± 1c	46.58 ± 0.9c	21.75 ± 0.93d	24.83 ± 0.4b	0.87	40.02 ± 0.59a	6.33 ± 0.5a
	Tamar	1.75 ± 0.2d	55.57 ± 0.7d	26.93 ± 0.06b	28.64 ± 0.8c	0.94	22.41 ± 0.53e	6.51 ± 0.3c
Angoor	Khalal	18.22 ± 0.3a	41.74 ± 0.3a	19.92 ± 0.17b	21.82 ± 0.3a	0.91	55.03 ± 0.18h	5.81 ± 0.1abc
	Rutab	8.50 ± 0.7a	49.84 ± 0.2b	24.58 ± 0.06b	25.26A ± 1b	0.97	28.03 ± 0.29g	6.15 ± 0.2b
	Tamar	0	59.36 ± 0.9a	30.04 ± 0.02a	29.32 ± 0.5ab	1.02	14.86 ± 0.21i	6.73 ± 0.2abc

Table 3. Antiradical efficiency, total phenolic contents, specific activity of CAT and POD and soluble protein contents at different fruit developmental stages.

Cultivars	Ripening stage	AE	TPC	CAT	POD	Protein
	Khalal	1.98 ± 0.03g	281.63 ± 1.53d	2.33 ± 0.41c	1.97 ± 0.16d	5.56 ± 1.48abc
Zehdi	Rutab	1.49 ± 0.03f	154.94 ± 0.29c	1.33 ± 0.16d	1.56 ± 0.05c	4.28 ± 0.85a
	Tamar	1.89 ± 0.02c	119.64 ± 0.97b	0.04 ± 0.06e	1.14 ± 0.03bc	3.21 ± 0.12a
	Khalal	2.80 ± 0.01e	258.96 ± 1.45e	1.49 ± 1.42g	1.60 ± 0.17de	5.31 ± 2.82cde
Be-Rehmi	Rutab	2.04 ± 0.01d	140.89 ± 0.49e	1.19 ± 0.70e	1.15 ± 0.20d	3.91 ± 0.21a
	Tamar	1.84 ± 0.02c	43.70 ± 0.03g	0.04 ± 0.31e	1.06 ± 0.39bcd	3.10 ± 0.90a
	Khalal	3.02 ± 0.01c	201.81 ± 0.67g	2.70 ± 0.34b	2.40 ± 0.37c	5.46 ± 0.2bcd
Neelum	Rutab	2.94 ± 0.01a	155.91 ± 0.86c	1.72 ± 0.12c	2.17 ± 0.15b	3.83 ± 0.35a
	Tamar	2.55 ± 0.02a	80.94 ± 0.24e	0.57 ± 0.31e	0.86 ± 0.12cd	2.91 ± 0.77a
	Khalal	1.79 ± 0.01h	354.54 ± 1.19a	1.80 ± 0.61e	2.86 ± 0.19b	5.43 ± 0.91bcde
Ko-Harba	Rutab	1.61 ± 0.01ef	163.07 ± 1.44b	2.03 ± 0.47b	2.28 ± 0.29b	3.74 ± 0.72a
	Tamar	1.27 ± 0.01e	26.93 ± 0.23h	1.77 ± 0.24a	1.25 ± 0.19b	3.04 ± 0.23a
	Khalal	3.25 ± 0.02a	232.72 ± 2.48f	1.48 ± 0.6g	1.79 ± 0.44de	5.18 ± 0.87e
Kozan Abad	Rutab	2.96 ± 0.01a	174.44 ± 1.51a	1.40 ± 0.21d	1.49 ± 0.41cd	4.62 ± 0.32a
	Tamar	2.13 ± 0.02b	148.67 ± 0.18a	1.33 ± 0.15b	1.15 ± 0.33b	3.25 ± 0.54a
	Khalal	1.69 ± 0.01i	339.39 ± 1.37b	2.97 ± 0.12a	3.79 ± 0.66a	5.75 ± 0.03a
Karblain	Rutab	1.24 ± 0.03g	145.83 ± 2.12d	2.05 ± 0.19b	2.95 ± 0.49a	4.78 ± 0.7a
	Tamar	1.27 ± 0.02e	151.63 ± 1.54a	1.08 ± 0.24d	1.79 ± 0.13a	3.04 ± 0.4a
	Khalal	3.10 ± 0.01b	117.09 ± 1.48h	2.18 ± 0.22d	1.78 ± 0.46de	5.61 ± 0.74ab
Jan-Sohar	Rutab	2.61 ± 0.03b	57.31 ± 0.85g	1.39 ± 0.29d	1.40 ± 0.39cd	4.57 ± 0.54a
	Tamar	1.39 ± 0.01d	107.63 ± 0.86c	1.20 ± 0.29c	0.78 ± 0.20d	3.07 ± 0.32a
	Khalal	3.01 ± 0.02c	275.81 ± 2.82d	2.30 ± 0.17c	1.43 ± 0.44e	5.43 ± 083bcde
Khadrawy-I	Rutab	2.23 ± 0.01c	102.54 ± 1.27f	0.82 ± 0.13f	0.69 ± 0.15e	4.35 ± 0.85a
	Tamar	1.87 ± 0.03c	50.28 ± 0.31F	1.41 ± 0.45b	0.43 ± 0.03e	3.13 ± 0.12a
	Khalal	2.47 ± 0.02f	312.36 ± 3.71c	2.13 ± 0.41d	1.79 ± 0.37de	5.22 ± 0.87de
Khadrawy-II	Rutab	2.25 ± 0.01c	144.05 ± 0.45de	1.42 ± 0.19d	1.26 ± 0.21cd	3.95 ± 0.35a
	Tamar	1.93 ± 0.03c	89.49 ± 0.62d	1.80 ± 0.4a	1.78 ± 0.12a	3.01 ± 0.03a
	Khalal	2.93 ± 0.03d	281.08 ± 1.01d	1.67 ± 0.14f	1.46 ± 0.85e	5.50 ± 0.7abc
Angoor	Rutab	1.74 ± 0.02E	160.65 ± 1.54b	2.65 ± 0.29a	2.81 ± 0.23a	4.07 ± 0.23a
	Tamar	1.20 ± 0.03e	51.15 ± 0.55f	1.40 ± 0.13b	1.06 ± 0.13bcd	3.47 ± 0.13a

Discussion

There are more than 5000 cultivars being cultivated all around the date producing countries and each cultivar is characterized by distinction in their color, morphological features, biochemical properties, and genetics. In morphological characterization, our findings are in good agreement with those already reported by Amorós *et al.,* (2009) and Guido *et al.,* (2011). DMR test also proposed significant differences ($p<0.05$) in the weights (fruit and pulp) of all the cultivars during the last three different maturation phases. So, Jan-Sohar and Khadrawy-II showed maximum values, while lower values were recorded in cv. Angoor (Table 1). Generally, the higher fruit dimensions were recorded at the khalal stage of date fruit. Similar researches on the fruit and pulp weights were carried out on Tunisian dates (Guido *et al.,* 2011) and Iranian dates (Rastegar *et al.,* 2012), which showed agreement with our results. The differences in final values are due to locality, genetics, and environment. The fruit/pulp ratio is the quality criteria for fruit characterization, as the level of ratio determines the better quality of date fruit. During the ripening, pulp represents (83.59–92.44%) of total fruit weight during ripening process (Table 1). Therefore, cvs. Jan-Sohar and Be-Rehmi seem to be the best at khalal, while Khadrawy-II is best during all three ranging khalal – tamar stage of date fruit development. This ratio was almost similar to the well-known Tunisian cultivar "*Deglet Nour*" that was equal to 90.54% (Guido *et al.,* 2011).

The proximate analysis was categorized into three different parameters, including moisture contents, pH and soluble protein contents based on the chemical properties. Moisture contents were high at the khalal stage and decreased to lower concentrations at tamar stage during the date fruit maturation process, which suggested that all the examined cultivars are as semi-dry cultivars. Besides, the pH values were elevated from khalal to rutab, and then up to the tamar stage during developmental phase. These findings strongly resemble with that of Tafti & Fooladi, (2006) and Rastegar *et al.,* (2012). However, the minor differences are may be due to cultivar type, and environmental and experimental conditions. Moreover, soluble protein contents decreased progressively from khalal to tamar stage during date fruit development. Amira *et al.,* (2011) reported that the protein contents increased during the ripening process, contradicting with our findings. Though, it has already been reported that as the free radical scavenging system declines, it degrades the protein, pigments and phospholipids level during plant tissue senescence (Prochazkova *et al.,* 2001). Elleuch *et al.,* (2008) reported 2.10% and 3.03% of protein contents for Tunisian famous *Deglet Nour* and *Allig* cultivar, which supported our findings. The contradiction in values and trend is may be due to the difference in analytical method, geographic origin, and climatic conditions.

The amount of glucose and fructose started accumulation from the khalal stage as their detected amount was low and then found in higher concentrations at tamar stage. Contrarily, the sucrose was higher at khalal stage and then degraded into reducing sugars, showing very little quantity at tamar stage. This degradation of sucrose is may be due to a sharp increase in an invertase enzyme activity, which can possibly invert sucrose into glucose and fructose (Guido *et al.,* 2011; Haider *et al.,* 2013). Amorós *et al.,* (2009) proposed that accumulation of reducing sugars is initiated from the khalal stage and reached into final concentrations at tamar stage, which proposed that dates are the excellent and readily available source of carbohydrates. Generally, sucrose underwent complete hydrolysis when moisture contents are decreased to a low level (Rastegar *et al.,* 2012; Haider *et al.,* 2014). Finally, the glucose/fructose ratio is of much importance because fructose is much sweeter than glucose, especially important for diabetic or diet conscious person. This ratio observed in our findings was < 1 in general, our results are in good agreement with other researchers on different date cultivars (Sahari *et al.,* 2007; Al-Farsi & Lee, 2008; Elleuch *et al.,* 2008).

TPC values of date fruits showed a gradually decreasing trend in our findings. Saafi *et al.,* (2009) reported that TPC values vary from 209–448 mg GAE/100 g FW in Tunisian date cultivars. Several other researchers also reported that TPC composition of date fruits decreased because of their oxidation by polyphenol oxidase enzyme as date fruits pass through progressive maturation stages (Al-Turki *et al.,* 2010; Awad *et al.,* 2011). The TPC level and their decreasing trend showed similarity with Saudi Arabian (Al-Turki *et al.,* 2010) and Tunisian (Amira *et al.,* 2012) date cultivars during fruit ripening process. The dissimilarities in TPC values are may be due to cultivar type, genetics, cultural practices, amount of sunlight received and environmental conditions.

Free radical are high reactive unchained molecules, often generated as the byproduct of biological reactions. The results of AA in Pakistani cultivars have demonstrated that AA was detected higher at khalal and gradual alteration was observed from rutab to tamar stage during fruit development and are in good agreement as already reported by Awad *et al.,* (2011). Generally, our statement that AA was higher at the khalal stage and lower at tamar stage is also supported by Amorós *et al.,* (2009) and Mansouri *et al.,* (2005). In fact, positive correlation between AA and TPC revealed the decrease in their quantity during the fruit maturation stages (Awad *et al.,* 2011). These variations in the values may reveal differences in cultivars, cultural operations, the quantity of fertilizer applied and different analytical approaches for the quantification of antioxidant activity.

In our findings, the specific activity of CAT was decreased during progressive developmental stages, except in Khadrawy-I and Khadrawy-II, where CAT activity was enhanced at tamar stage. In general, Karblain possessed the higher composition of CAT and POD during the fruit ripening phase. Haider *et al.,* (2014) and Awad *et al.,* (2011) estimated the CAT and POD activity of different date palm cultivar during the fruit maturation process and demonstrated similar trend and values with our findings. The decrease in the activity of CAT and POD enzymes was due to a decrease in AA and TPC during the ripening process. The difference in cultivar, genetics, cultural practices and environmental conditions may affect the final composition of the date fruit antioxidant enzyme activity.

Conclusion

The morphological parameters of selected date fruit cultivars commonly grown in Pakistan during the different developmental phases showed diversity in shape and weight, but fruit/pulp ratio accounted for (83.59–92.44%) in all the cultivars. Moreover, these cultivars are good source of protein, low moisture contents, but possess high reducing sugar (glucose and fructose) contents especially at tamar stage. On the other hand, the amount of TPC, AA, CAT, and POD was higher at the khalal stage and then were reduced at tamar stage, revealing the importance of khalal stage as the potential stage for nutrition. Therefore, our findings proposed that Pakistani date cultivars can compete with the important world marketed date varieties. Hence, consumers could take these cultivars into consideration.

Acknowledgement

Author(s) acknowledge the financial assistance of International Center for Development and Decent Work (ICDD), Germany.

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(Received for publication 13 August 2017)