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Effects of the Ripening Stage on Phenolic Profile, Phytochemical Composition and Antioxidant Activity of Date Palm Fruit

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ABSTRACT: Four cultivars (*Gondi*, *Gasbi*, *Khalt Dhahbi*, and *Rtob Ahmar*) of Tunisian date palm (*Phoenix dactylifera* L.) fruits at 3 maturation stages, *besser*, *rutab* and *tamr*, were analyzed for their antioxidant activities (AA) using 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid) and 2,2-diphenyl-1-picrylhydrazyl radicals cation, and reducing power (RP) methods. The total phenolic (TPC), total flavonoid (TFC), and condensed tannins (CTC) contents were measured. Results showed that all samples have the highest TPC, TFC, CTC, and AA at *besser* stage. A significant correlation ($p < 0.05$) was found between TPC, TFC, CTC and AA during ripening. Sixteen phenolic compounds were identified and quantified by HPLC. The major ones were caffeic, ferulic, protocatechuic, and catechin for the majority of cultivars. Our data indicate that common date fruits are rich in natural antioxidants that might be more widely used by both the general population and the food industry as a source of bioactive human health promoter phytochemicals.

KEYWORDS: *date fruits, maturation stage, phytochemical composition, antioxidants' activity, phenolic profile*

INTRODUCTION

Free radicals play an important role in some pathogenesis of serious diseases, such as neurodegenerative disorders, cancer, liver cirrhosis, cardiovascular diseases, atherosclerosis, cataracts, diabetes, and inflammation.[1] Compounds that can scavenge free radicals have great potential in the reduction of disease risk. Due to the presence of the conjugated ring structures and hydroxyl groups, many phenolic compounds (such as phenolic acids, flavonoids, and tannins), ascorbic acid, tocopherols, and carotenoids, can potentially function as antioxidants by scavenging superoxide anion, singlet oxygen, and lipid peroxyl radicals, by stabilizing free radicals involved in oxidative processes through hydrogenation or combining with oxidizing species.[2]

Various factors (climatic, agronomic, genomic, pre- and postharvest conditions, and processing) may affect the chemical composition of plant foods and may have a significant role in determining the phenolic composition and the bioactivity of these compounds.[3] Maturity stage is another important factor that may influence the compositional quality of fruit and vegetables. In fact, during fruit ripening, several biochemical, physiological, and structural modifications happen and these changes determine the fruit quality attributes. Harvesting at the proper maturity stage is essential for optimum quality and often for the preservation of this quality after harvest and storage.

Date palm is the most successful and important subsistence crop in most of the hot arid desert regions. The sweetness, mouthfeel, and texture of date fruit is closely related to the maturity and ripeness stage of the date. Generally, whole dates are harvested and marketed at three stages of development: mature firm (*besser or khalal*), half-ripe (*rutab*), and ripe (*tamr*). The decision for harvesting at one or other stage depends on cultivar (cv) characteristics, especially soluble tannins and sugar contents, climatic conditions, and market demand.[4] Once ripened, dates have a short shelf life, which may reflect lower levels of antioxidants. The composition of the antioxidant phenolics in fruit changes dramatically during ripening, and this can affect shelf life.[5] A considerable amount of work has been carried out on the changes in the physical and chemical characteristics of date palm fruit during growth and development.[6,7] However, little publications are available on the total phenolic (TPC),[8−10] condensed tannin (CTC) contents,[11] and antioxidant activity (AA)[8−10] of date fruit during maturation stage. However, no study was reported on the variation of the total flavonoid content (TFC) and the profile of phenolic compounds during ripening. In the current study, the TPC, TFC, CTC, the AA and the profile of phenolic compounds of four common date palm cultivars grown in Kébili region in southern Tunisia at three maturation stages, namely *besser*, *rutab*, and *tamr* are evaluated.

MATERIALS AND METHODS

Plant Material. Fresh fruit sample used in these experiments consisted of four cultivars (cvs) of dates (Table1) and were procured from Kébili, southern Tunisia from the 2008 harvest season at three different maturation stages according to the pollination date: *besser*, *rutab*, and *tamr* stages. Immediately after harvesting, at each maturation stage, date fruits were selected (for freedom from defects and color uniformity) and stored at −20 °C until analysis. Three replicates were carried out and 10 dates were used for each replicate for each type of date.

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Table 1. Local Arabic Names, Ripening Time at *tamr* Stage, Color at Various Maturation Stages, and the Eating Quality of the Four Common Tunisians Cultivars Reported in this Study

local arabic name of the cultivars[a]	ripening time (month)	color at *Besser*	color at *Rutab*	color at *Tamr*	eating quality
Gasbi	August	yellow apricot	light amber	dark amber	good
Gondi	September	straw yellow	translucent honey	translucent amber	very good
Khalt Dhabi	November	yellow to golden	amber	dark amber	fair
Rtob Ahmar	October	yellow with light red nuance	amber	dark amber	good

[a]Local arabic name of cultivars are arranged alphabetically.

Chemicals and Reagents. All chemical, standards including phenolic acids and reagents used were purchased from Sigma−Aldrich Co. Ltd. (St. Louis, MO, U.S.).

Extraction Method. Fruit extracts for TPC and AA analysis were prepared according to the method of Al-Farsi et al.[12] Briefly, sample (200 mg) was extracted with 2 mL of 50% methanol for 2 h at room temperature on an orbital shaker set at 200 rpm. After centrifugation at 1000 ×*g* for 15 min, the supernatant was decanted and the pellets were extracted under identical conditions. Supernatants were combined and used for total phenolic assay and AA. Fruit extracts for TFC and CTC analysis were prepared using 100% methanol under identical conditions.

Phytochemical Composition. *Total Phenolic Content.* TPC were estimated using Folin-Ciocalteu reagent as described by Al-Farsi et al.[12] Measurements were carried out in triplicate and calculations based on a calibration curve obtained with gallic acid. The TPC was expressed as mg of gallic acid equivalents (GAE) per 100 g of fresh weight (FW).

Total Flavonoid Content. TFC were determined according to the method of Zhishen et al.[13] An aliquot (250 μL) of each extract or standard solution was mixed with 1.25 mL of dd H_2O and 75 μL of 5% $NaNO_2$ solution. After 6 min, 150 μL of 10% $AlCl_3{\cdot}H_2O$ solution were added. After 5 min, 0.5 mL of 1 M NaOH solution were added and then the total volume was made up to 2.5 mL with distilled H_2O. Following thorough mixing of the solution, the absorbance against blank was determined at 510 nm. (+)-Catechin was utilized for constructing the standard curve (0.05−0.5 mg/mL). The results were expressed as mg catechin equivalents (CE)/100g FW.

Condensed Tannins Content. CTC were determined according to the method of Julkunen−Titto.[14] An aliquot (50 μL) of each extract or standard solution was mixed with 1.5 mL of 4% vanillin (prepared with methanol) and then 750 μL of concentrated HCl were added. The well-mixed solution was incubated at ambient temperature in the dark for 20 min. The absorbance against blank was read at 500 nm. (+)-Catechin was used to make the standard curve (0.05−1 mg/mL). The results were expressed as mg CE/100g FW.

Antioxidant Activities. *DPPH Assay.* The AA was determined using the DPPH· test according to the methods of Brand-Williams et al.[15] and Mansouri et al.[16] Different dilutions of the phenolic extract were prepared for each cultivar. An aliquot of 25 μL of a diluted sample was added to 975 μL DPPH· solution (6×10^{-5} M) and vortexed. The decrease in absorbance was determined at 515 nm recorded at 1 min, 5 min, and then every 5 min until the reaction reached the plateau, using a UV spectrophotometer. The DPPH· concentration in the reaction medium was calculated from the calibration curve, as determined by linear regression:

$$A_{515\text{nm}} = 5.816 \times [\text{DPPH}^{\bullet}](\mu\text{g/mL}) - 0.043 (R^2 = 0.996)$$

Different concentrations (expressed as the number of antioxidant μg per μg of DPPH) were used, and for each cultivar, the reaction kinetics was plotted. From these graphs, the percentage of DPPH· remaining at the steady state was determined. These values were transferred onto another graph showing the percentage of residual stable radical at the steady state as a function of the mass ratio of antioxidant to DPPH. The latter was used to determine the efficient concentration (EC_{50}), which is the amount of antioxidant necessary to decrease the initial DPPH concentration by 50%. For reasons of clarity, we will speak in terms of antiradical efficiency (AE = $1/EC_{50}$). The antioxidant was more efficient where the AE was larger.

ABTS Assay. AA was measured using an improved ABTS method as described by Re et al.[17] The ABTS radical cation ($ABTS^{\cdot+}$) 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 12 to 16 h, the $ABTS^{\cdot+}$ solution was diluted with 80% ethanol to obtain an absorbance of 0.700 ± 0.002 at 734 nm. An aliquot of 25 μL of the test sample was added to 975 μL of $ABTS^{\cdot+}$ solution and vigorously mixed. The absorbance at 734 nm was recorded every 20 s for 6 min. A standard curve was obtained by using Trolox standard solution at various concentrations (ranging from 0 to 2 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.[17]

Reducing Power. In the reducing power assay, the yellow color of the test solution changes to various shades of green and blue, depending on the reducing power of each extract. The presence of reducers (i.e., antioxidants) causes the reduction of the Fe^{3+}/ferricyanide complex to the ferrous form. Therefore, Fe^{2+} concentration can be monitorized by measuring the formation of Perl's Prussian blue at 700 nm. The capacity of date extracts to reduce Fe^{3+} was assessed by the method of Oyaizu.[18] An aliquot of each sample or standard solution prepared with methanol (250 μL) was mixed with 250 μL of sodium phosphate buffer (0.2 M, pH 6.6) and 250 μL of 1% $K_3Fe(CN)_6$ incubated at 50 °C for 20 min. After adding 250 μL of 10% trichloroacetic acid, the mixture was centrifuged at 3750 ×*g* for 10 min. The supernatant (100 μL) was then taken out and immediately mixed with 100 μL of methanol and 25 μL of 0.1% ferric chloride. After incubation for 10 min, the absorbance against blank was determined at 700 nm. The EC_{50} value is the concentration at which the absorbance is 0.5.

Identification and Quantification of Phenolic Compounds. *Preparation of Samples for HPLC Analysis.* Date fruit dissolved in HPLC grade methanol, to make 10 mg/mL sample solution, was shacked overnight at room temperature. After centrifugation, all samples were filtered with a 0.45 μm membrane filter before HPLC analyzes.

HPLC Analysis. The phenolic compounds' analysis was carried out using an Agilent Technologies 1100 series liquid chromatography (HPLC, Palo Alto, CA) coupled with a UV−vis multiwavelength detector. The separation was carried out on a 250 × 8 mm, Eurosphere 100−5, C_{18} column at ambient temperature. The mobile phase consisted of acetonitrile (solvent A) and water with 0.2% sulphuric acid (solvent B). The flow rate was kept at 0.5 mL min^{-1}. The gradient program was as follows: 15% A/85% B, 0−12 min; 40% A/60% B, 12−14 min; 60% A/40% B, 14−18 min; 80% A/20% B, 18−20 min; 90% A/10% B, 20−24 min; and 100% A, 24−28 min.[19] The injection volume was 20 μL, and peaks were monitored at 280 nm. Peaks were identified by congruent retention times compared with standards (gallic, protocatechuic, syringic, 3-hydroxybenzoic, isovanillic, chlorogenic, caffeic, *p*-coumaric, *m*-coumaric, *o*-coumaric, ferulic, cinnamic, apigenic, catechin, hydroxyphenylacetic, and phenylacetic acids). Analyzes were performed in triplicate.

Statistical Analysis. Data are reported as the mean ± SD of three replicates of ten fruits each per stage. Statistical analysis was performed with SPSS 11.0 (SPSS Inc., Chicago, IL, U.S.). The data were analyzed by one way analysis of variance (ANOVA). Duncan's test ($p < 0.05$)

was used to determine significant differences between means and Pearson's test was employed to perform the correlation analysis.

RESULTS AND DISCUSSION

Phytochemical Composition. The TPC, TFC, and CTC in the four common date cultivars at three maturity stages are presented in Figures 1 and 2. Significant differences ($p < 0.05$)

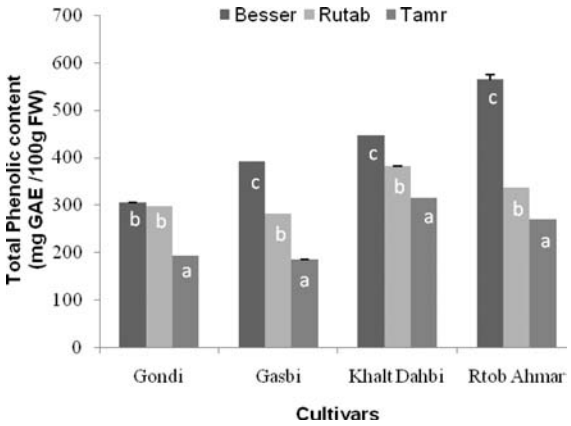

Figure 1. Total phenolic content of date cultivars during fruit ripening. Data are reported as the mean ± SD of three replicates of ten fruits each per stage. For each cultivar, bars not sharing a common superscript letter differ significantly at $p < 0.05$ by Duncan's test.

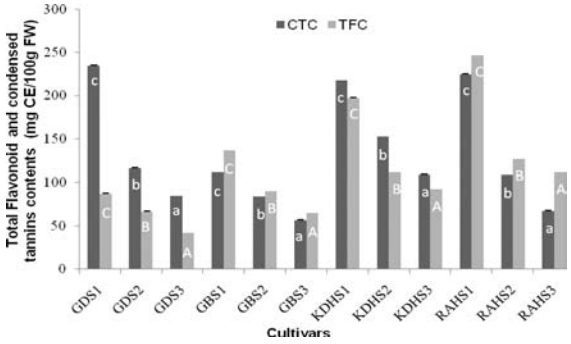

Figure 2. Total flavonoid (TFC) and condensed tannins (CTC) contents expressed as milligrams catechin equivalent/100 g fresh weight (mg CE/100g Fw) of date cultivars during ripening. Data are reported as the mean ± SD of three replicates of ten fruits each per stage. Parts a, b, and c show significant differences for CTC between maturation stages at $p < 0.05$ by Duncan's test. Parts A, B, and C show significant differences for TFC between maturation stages at $p < 0.05$ by Duncan's test. GD; *Gondi*, GB; *Gasbi*, KDH; *Khalt Dhabi*, RAH; *Rtob Ahmar*. S1; *Besser*, S2; *Rutab*, S3; *Tamr* stages.

in the levels of phenolics, flavonoids, and condensed tannins were found between cultivars and between stages among the same cultivar. At the *besser* stage, all of the four date cultivars displayed the highest level of TPC where *Rtob Ahmar* had the important amount (563.71 mg GAE/100 g FW) and *Gondi* the lowest amount (304.24 mg GAE/100 g FW). However, at both *rutab* and *tamr* stages, *Khalt Dhahbi* cv had the highest TPC (380.81 and 314.39 mg GAE/100 g FW, respectively). TPC decreased significantly ($p < 0.05$) from *besser* to *tamr* stage (Figure 1). The decrease in phenolic contents can be explained by the decline of phenolic substances (generically referred to as tannins) as the dates matured to *tamr* stage.[6] In other way, the decline in the total phenolic level may be due to the oxidation of phenolic content by polyphenol oxidase that characterizes these stages of maturity.[20] The decreases of TPC in date fruit was also reported in other works.[8−10] Comparing our results with those stated by these authors we found that Tunisian common dates contained high amount of TPC. These differences may be due to the fact that the fruit phenolic contents can be affected by many factors such as the variety, cultivation, area, ripeness, harvesting time, climatic conditions, storage time, and environment factors.

Our results showed that total flavonoid content was dependent on the maturity stage and gradually decreased as maturity proceeded (Figure 2). The order of TFC in date fruit till full maturation is: *Rtob Ahmar* > *Khalt Dhabi* > *Gasbi* > *Gondi*. The highest content (87.34 to 246.83 mg CE/100g FW) was recorded at *besser* stage and the lowest content (41.77 to 111.39 mg CE/100g FW) was detected at the *tamr* stage. Same deduction was also found in nectarine fruits.[21] Our results are in agreement, especially at *tamr* stage, with those found by Chaira et al [22] for *Korkobbi* cv (54.46 mg quercetin equivalents/100 g FW). In contrary, are different to those reported by Al-Humaid et al.[23] for three Saudi Arabia common dates called *Sukkari*, *Nabtat Ali*, and *Rashudia*, their TFCs were 11.30, 14.70, and 17.10 mg QE/100 g of sample, respectively. These differences may be due to cultivar type, geographical origin, growing and environmental conditions (mainly light and temperature) during fruit development, harvest time, and experimental and storage conditions.

It is worth noting that dates contain tannins below the skin of drupe, made mainly of polyphenols, and two groups of them (phenolic acids and condensed tannins) were thought to be important in producing the astringent sensory response.[6] Tannins are well-known as potent antioxidants. The soluble tannins are gradually converted into nonsoluble condensed form, known as procyanidins, as the fruit begins to ripen and advances progressively, thus losing its astringent taste and becoming more palatable. Comparing the three maturation stages we found that *besser* stage was characterized by the greatest level of CTC. In this stage, *Gondi* cv. contained the highest level (234.70 mg CE/100 FW). This result confirms the astringent taste of this cultivar. In the contrary *Gasbi* cv. contained the lowest CTC (111.11 mg CE/100 FW), which may justify the slight astringent taste of this cultivar at *besser* stage. The content of condensed tannins, in the four cultivars, decreased significantly ($p < 0.05$) during ripening (Figure 2). Myhara et al.[11] reported that *khalas* cv. contained 280 and 150 mg tannins per 100g FW at *kimiri* (early greenish stage) and *tamr* stages, respectively. Furthermore, the study reported by Tafti and Fooladi,[24] established that Mozfati date fruit presented a level of tannins varied from 1.3% at *Kimiri* stage to 0.23% at *Tamr* stage. These results show a corresponding decrease in astringency.

Antioxidant Activity. The AA determined by DPPH, ABTS, and Reducing power (RP) methods in the four common date cvs at the three different ripening stages are shown in Figure 3. Disregarding the analytical method used, for all date cvs, the highest AA value was estimated in date fruits at the *besser* stage and the lowest value was recorded at the full ripe stage. Thus, the AA exhibited a significant ($p < 0.05$) decrease during fruit ripening. These results agree with those found by Awad.[10] In comparing the AA of the four cvs measured by the three testes, we found that both *Khalt Dhabi and Rtob Ahmar* cvs have the highest activities during all the maturation stages. Few works are reported on the AA of date palm fruit according to maturation stage.[8,9] These works showed that date palm have a very noticeable AA expressed as mmol TE/100 g FW at *besser* stage.

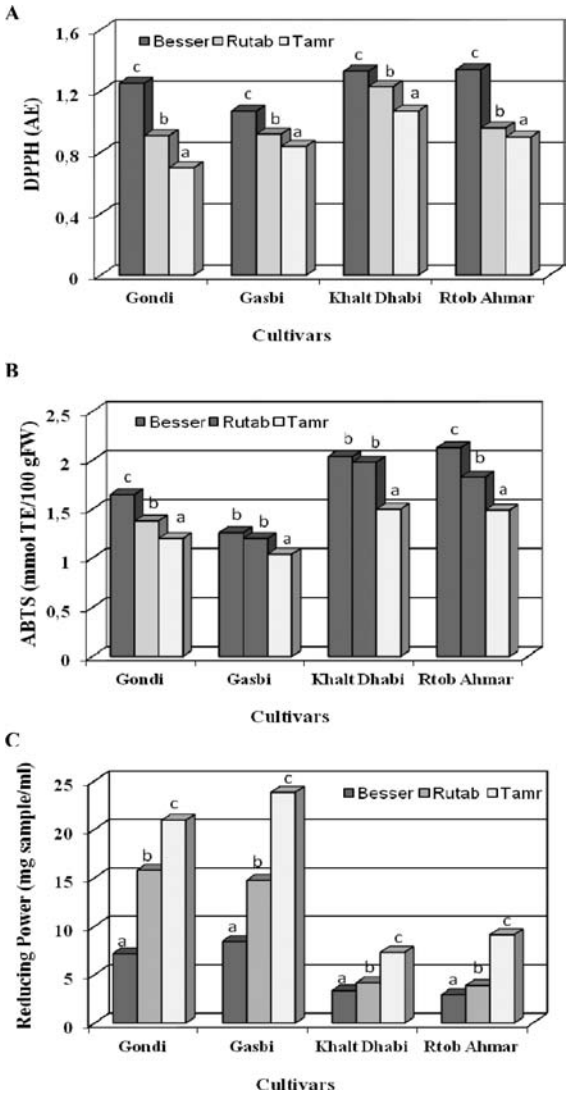

Figure 3. Antioxidant activity of three cultivars of date fruits during ripening evaluated with DPPH (A), ABTS (B), and RP (C) tests. Data are reported as the mean ± SD of three replicates of ten fruits each per stage. For each cultivar, a, b, and c refer to significant differences between maturation stages at $p < 0.05$ by Duncan's test.

Saafi et al.[25] evaluated the AA of four Tunisian ripe date fruit cvs by the DPPH and ABST methods and found a similar AA to those found in our study. A study reported by Biglari et al.[26] showed that Iranian date palm fruits have a low level of TEAC (22.83 ± 1.49 to 54.61 ± 5.19 μmol TE/100g FW) except for *Kharak* sample with 500.33 ± 47.17 μmol TE/100g FW. Furthermore, Vinson et al. [27] used the inhibition of lower density lipoprotein oxidation ($1/IC_{50}$) method to evaluate the AA of American date fruits and reported a value of 2.17 μmol TE. These results are different to our. This may reflect differences in cultural practices and climatic conditions among locations, including differences in temperature, or water stress, or mineral nutrient availability. The soil types as well as fertilization parameter also influence the nutritional composition and antioxidant activity by affecting the water and nutrient supply to the plant of the harvested fruit.

Correlation between Phytochemical Compounds and Antioxidant Activities. In order to assess the contribution of TPC, TFC, and CTC to the total antioxidant activity of the pulp of date fruits, the Pearson's correlation coefficient was calculated. A highly positive relationship was found between antioxidant capacity TEAC values and contents of antioxidant components (total phenols, $r = 0.848$; total flavonoids, $r = 0.767$ and condensed tannins, $r = 0.891$, $p < 0.01$), and so did that between AE values of DPPH radical-scavenging activity and contents of antioxidant components (total phenols, $r = 0.811$; total flavonoids, $r = 0.769$ and condensed tannins, $r = 0.770$, $p < 0.01$). A significant correlation ($p < 0.01$) was also observed between EC_{50} values of reducing power and TPC ($r = -0.806$), TFC ($r = -0.735$) and CTC ($r = -0.661$). The strong correlation found between the antioxidant capacity and total phenols, total flavonoids, and condensed tannins content suggests a strong contribution of these compounds in the total antioxidant capacity and healthiness of dates. Several studies have reported positive correlations between antioxidant capacity and total phenol content in dates[16,8,10] and in other fruits.[28]

Identification and Quantification of Phenolic Compounds. To the best of our knowledge, phenolic acid profiles of date fruit during ripening are still unknown. This is the first study that examines the phenolic profile of date fruit during ripening. The HPLC analysis of date pulp methanolic extracts revealed the presence of 16 phenolic compounds (Table 2). Five hydroxybenzoic acids (HBA), including gallic, protocatechuic, syringic, isovanillic and 3-hydroxybenzoic acids, seven hydroxycinnamic acids (HCA), including, chlorogenic, caffeic, ferulic, *p*-, *m*-, and *o*-coumaric, and cinnamic acids, two flavonoids, including catechin and apigenin, as well as two other phenolic acids; hydroxyphenylacetic and phenylacetic acids, were identified and quantified in the date fruits (Table 2). Hydroxyphenylacetic and phenylacetic acids were not described before in date cultivars, being detected news. A significant differences ($p < 0.05$) was found in the phenolic compounds amounts between maturation stages for the majority of cultivars. Among the identified compounds, gallic, syringic, caffeic, ferulic, *m*- and *o*-coumaric, phenylacetic acids, and catechin were present in all cultivars until full maturation. Cinnamic, *p*-coumaric, 3-hydroxybenzoic, apigenic, and hydroxyphenylacetic acids were not detected only in *Gondi* cv. at the three stages. From the groups of phenolic compound analyzed, the hydroxycinnamic acids (sum of 16.75 to 19.40 mg/100g FW) were the most predominant group at all stages of ripening. These results concord with those found in other fruits,[29] and in Deglet Nour syrup extracts.[30] Ferulic acid followed by caffeic acid were clearly the most abundant phenolic acids found in the four cvs, with a highest quantities varying from 3.48 to 5.96 and from 3.04 to 5.75 mg/100g FW during ripening, respectively. These two HCAs were found at important levels at *besser* stage. A *p*-coumaric acid was also found at an important level at *besser* stage but only in three cultivars. The presence of ferulic and *p*-coumaric acids in Tunisian date fruit was confirmed by a recent study reported by Mrabet et al.,[31] when ferulic acid was the most abundant phenol compound with a level ranged between 14.35 to 56.35 mg/100 g fiber, and *p*-coumaric was the minor one detected in eleven matured date fruits. In another way, two HBAs were found with an appreciate amount until maturation, syringic acid (1.33−4.95 mg/100g FW) in all of the four cvs and protocatechuic acid (3.241- 4.41 mg/100g FW) not detected only in *Khalt Dhabi* cv. at *besser* and *tamr* stages. However, gallic acid was found with inferior levels (0.70−2.364 mg/100g FW) in all of the cvs. The HBAs are generally minor phenolics in edible plants, but are often observed as

Table 2. Phenolic Acids Contents (mg/100g FW) of Four Common Tunisians Date Fruits at Three Ripening Stages[a,b]

	Gasbi			*Gondi*			*Khalt Dhahbi*			*Rtob Ahmar*		
	Besser	*Rutab*	*Tamr*	*Besser*	*Rutab*	*Tamr*	*Besser*	*Rutab*	*Tamr*	*Besser*	*Rutab*	*Tamr*
GA	1.00 ± 0.01a	1.22 ± 0.09b	1.55 ± 0.01c	1.85 ± 0.08a	1.80 ± 0.02a	1.62 ± 0.05b	2.36 ± 0.04c	0.70 ± 0.01a	1.67 ± 0.04b	1.79 ± 0.06b	1.80 ± 0.01b	1.61 ± 0.01a
PCA	4.31 ± 0.02b	4.13 ± 0.02b	3.64 ± 0.03a	4.41 ± 0.03b	4.40 ± 0.01b	3.24 ± 0.01a	nd	3.49 ± 0.07b	nd	3.78 ± 0.09b	3.32 ± 0.01a	3.75 ± 0.02b
SA	3.05 ± 0.05a	3.77 ± 0.05b	3.97 ± 0.02c	4.15 ± 0.06a	4.95 ± 0.01b	4.20 ± 0.01a	3.32 ± 0.09c	1.90 ± 0.01b	1.33 ± 0.07a	3.53 ± 0.04c	2.99 ± 0.08b	2.46 ± 0.06a
3HBA	1.06 ± 0.04a	1.26 ± 0.03b	0.98 ± 0.01a	nd	nd	nd	nd	1.39 ± 0.01c	1.20 ± 0.02b	1.05 ± 0.02b	2.98 ± 0.07c	nd
IVA	nd[c]	nd	nd	3.97 ± 0.02c	3.44 ± 0.06b	nd	nd	3.04 ± 0.03b	3.13 ± 0.06c	3.13 ± 0.06c	2.00 ± 0.06a	2.18 ± 0.03b
CHA	3.50 ± 0.02c	3.16 ± 0.05b	3.06 ± 0.06a	nd	nd	3.90 ± 0.01b	nd	2.63 ± 0.05b	1.79 ± 0.05a	2.91 ± 0.02c	2.81 ± 0.02b	2.49 ± 0.02a
CFA	3.53 ± 0.01c	3.35 ± 0.04b	3.19 ± 0.03a	5.75 ± 0.08c	4.77 ± 0.01b	4.64 ± 0.05a	5.62 ± 0.09c	3.35 ± 0.03a	3.41 ± 0.02b	3.63 ± 0.02c	3.12 ± 0.03b	3.04 ± 0.03a
p-CMA	4.42 ± 0.01b	nd	nd	nd	nd	nd	5.44 ± 0.05a	3.30 ± 0.03a	3.96 ± 0.03b	3.86 ± 0.03b	3.86 ± 0.02b	3.78 ± 0.06a
FA	5.46 ± 0.05c	5.15 ± 0.01b	4.97 ± 0.06a	5.23 ± 0.02c	5.14 ± 0.04b	5.09 ± 0.03a	5.96 ± 0.02c	3.48 ± 0.07a	4.27 ± 0.05b	4.79 ± 0.09b	4.42 ± 0.04a	4.38 ± 0.04a
m-CMA	1.25 ± 0.09a	2.28 ± 0.04c	2.04 ± 0.08b	2.38 ± 0.03b	2.18 ± 0.05a	2.15 ± 0.01a	2.26 ± 0.06b	1.51 ± 0.06a	2.56 ± 0.03c	1.44 ± 0.08a	1.50 ± 0.04a	1.72 ± 0.04b
o-CMA	2.05 ± 0.04a	2.39 ± 0.09c	2.26 ± 0.06b	3.10 ± 0.03b	3.05 ± 0.04b	2.08 ± 0.01a	3.65 ± 0.07c	1.99 ± 0.07a	2.24 ± 0.02b	1.64 ± 0.03a	1.94 ± 0.07b	2.12 ± 0.02c
CNA	nd	0.66 ± 0.08a	1.51 ± 0.01b	nd	nd	nd	nd	0.56 ± 0.03b	0.60 ± 0.02b	nd	nd	nd
AGA	nd	1.52 ± 0.02b	0.92 ± 0.04a	nd	nd	nd	0.73 ± 0.08b	0.55 ± 0.03a	1.15 ± 0.03c	0.64 ± 0.04b	0.47 ± 0.02a	1.63 ± 0.02c
CTA	3.34 ± 0.06c	2.75 ± 0.05b	2.61 ± 0.02a	3.84 ± 0.02c	3.52 ± 0.02b	2.67 ± 0.03a	3.84 ± 0.05c	3.00 ± 0.01b	2.92 ± 0.07a	3.37 ± 0.01c	2.80 ± 0.08b	2.48 ± 0.02a
HPAA	1.56 ± 0.02b	nd	nd	nd	nd	nd	2.44 ± 0.04b	2.72 ± 0.08c	1.61 ± 0.01a	2.46 ± 0.09c	1.19 ± 0.01b	nd
PAA	1.71 ± 0.05b	1.49 ± 0.03a	2.13 ± 0.04c	1.85 ± 0.01c	1.24 ± 0.02a	1.61 ± 0.06b	2.02 ± 0.06a	2.01 ± 0.05a	2.43 ± 0.01b	1.49 ± 0.07a	1.42 ± 0.07a	1.84 ± 0.03b
∑HBA	9.43 ± 0.02a	10.39 ± 0.09c	10.14 ± 0.07b	14.38 ± 0.08b	14.59 ± 0.04b	9.06 ± 0.07a	5.69 ± 0.03a	10.52 ± 0.06c	7.34 ± 0.09b	13.30 ± 0.01c	13.11 ± 0.03b	10.00 ± 0.06a
∑HCA	20.23 ± 0.02b	17.01 ± 0.07a	17.05 ± 0.04a	16.46 ± 0.03b	15.14 ± 0.01a	17.86 ± 0.03c	22.95 ± 0.07c	16.84 ± 0.07a	18.84 ± 0.07b	18.29 ± 0.02b	17.66 ± 0.05a	17.54 ± 0.04a
∑PA	36.28 ± 0.05c	33.18 ± 0.06b	32.86 ± 0.07a	36.54 ± 0.02c	34.49 ± 0.09b	31.21 ± 0.06a	37.69 ± 0.07c	35.65 ± 0.07b	34.31 ± 0.04a	39.57 ± 0.03c	36.67 ± 0.06b	33.50 ± 0.04a

[a]Data are reported as the mean ± SD of three replicates of ten fruits each per stage. Means followed by the same letter in the same line (For each cultivar) are not significantly different ($p > 0.05$). GA; Gallic acid. PCA; Protocatechuic acid. SA; syringic acid. 3HBA; 3-Hydroxybenzoic acid. IVA; Isovanillic acid. CHA; Chlorogenic acid. CFA; Caffeic acid. *p*-CMA; *p*-coumaric acid. FA; Ferulic acid. *m*-CMA; *m*-coumaric acid. *o*-CMA; *o*-coumaric acid. CNA; Cinnamic acid. AGA; Apigenic acid. CTA; Catechin. HPAA. Hydroxyphenylacetic acid. PAA; Phenylacetic acid. [b]∑HBA: sum of hydroxybenzoic acids; ∑HCA: Sum of hydroxycinnamic acids; ∑PA: Sum of phenolic acid. [c]Not detected.

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components of complex structures, such as hydrolyzable tannins. In the present study, the concentration of HCA and HBA are inversely correlated ($r = -0.858$, $p < 0.01$). Although the higher quantity of HCA, the sum of their amount decreased from *besser* to *rutab* stage (19.40 to 16.75 mg/100g FW) followed by a slight increased until full ripening. This decrease may be due to the presence of free esters of phenolic acids,[12] together with their accumulation in insoluble form at the early stages of maturity (*besser* stage) suggest that they are progressively bound to the cell walls. The integration of phenolic esters into cell walls is an important mechanism by which plants defend themselves against pathogens and strengthen their cell walls.[32] Furthermore, the accumulation of these esters protects the cells against membrane damage caused by reactive oxygen spieces.[33] Regarding the flavonoid group, we found that catechin, a flavan-3-ols subgroup, was detected with the highest amount (3.34 - 3.84 mg/100g FW) at *besser* stage in all the cvs and then decreased as date matured. This decrease correlates with that of TFC ($r = 0.576$, $p < 0.05$). Catechin may be the dominant flavonoid in date fruit and contributes predominantly to their antioxidant activity. Among the flavonoids, the flavonols (catechin and proanthocyanidins) are considered good antioxidants due to the particular substitution pattern of free hydroxyl groups on the flavonoid skeleton. Hong et al. [34] studied the flavonoid glycosides and procyanidin composition of Deglet Nour cv. and identified several procyanidin oligomers and flavonoid glycosides of lutein, quercitin and apigenin. The non-identification of the majority of these compounds in our cultivars due essentially to the difference in the wavelength used. Other phenolic compounds were detected in these four cvs, hydroxyphenyl acetic and phenylacetic acids which the sum of their amounts decreased from 3.94 to 3.59 mg/100g FW as the date ripened.

A significant correlation was found between the sum of the phenolic acid amounts ($\sum$PA) identified by HPLC and the TPC determined by the Folin−Ciocalteu method ($r = 0.792$, $p < 0.01$). The $\sum$PA correlate also with TFC ($r = 0.746$, $p < 0.01$) and CTC ($r = 0.662$, $p < 0.05$). Consequently, the evolution of the phenolic compounds of the four cultivars during ripening explains the diminution in their TPC, TFC, and CTC and also explains the difference found between them. The highest level of their phenolic compounds at *besser* stage explains their astringent taste due mainly to catechin, gallic, and protocatechuic acids. These acids exhibit potent antiradical activity.[15] The phenolic profile of date fruit especially at *tamr* stage was previously reported by many studies. Al-Farsi et al.[12] identified four free phenolic acids (protocatechuic, vanillic, syringic, and ferulic acids) in Omani dates with total variance between 2.61 and 12.27 mg/100 g in fresh dates, and between 6.06 and 14.77 mg/100 g in their sun-dried counterparts. This study shows a smaller or similar concentration in phenolic acids. Regnalut−Roger et al.[35] studied phenolic acids in dried Tunisian ripe dates and found eight phenolic acids (gallic, protocatechuic, *p*-hydroxybenzoic, vanillic, caffeic, syringic, *p*-coumaric, and ferulic acids). Although this study showed a similar phenolic acids profile, the concentrations of phenolics was much higher than those reported earlier. Mansouri et al.[16] studied phenolic profiles of seven different cultivars of ripe date fruits grown in Algeria and found that all cultivars contained *p*-coumaric, ferulic and sinapic acids as well as some cinnamic acid derivatives, but these were not quantified. Three different isomers of 5-*o*-caffeoylshikimic acid were also detected. Many factors (such as location, genetic variability, environmental characteristics, and maturation stage) have been reported to influence the content and variability of phenolic compounds within the same fruit type. In another study, Karasawa et al.[36] identified six major polyphenol compounds in dried date extract and showed that chlorogenic, caffeic, ferulic acids, and pelargonin increased IFN-γ mRNA expression in mouse Peyer's Patch cell significantly compared with polyphenol-free, protocatechuic, and syringic acids. Moreover, chlorogenic, caffeic, and ferulic acids enhanced the number of IL-12$^+$CD11b$^+$ cells.[36] The predominant phenolics found in date fruits are very active as antioxidants,[37] and the antioxidant activity in dates was highly correlated to the phenolic contents.[16]

The results presented in this work clearly demonstrate that the amounts of phenolic compounds and the antioxidant capacities of date fruits were affected by maturation stages. Date cultivars have different levels of TPC, TFC, CTC, and AA during ripening most of which were present at *besser* stage. A significant variability and a positive correlation were found in phenolic contents and antioxidant capacity in all assessed cultivars at the three maturation stages. It was shown that most cultivars have a notable antioxidant activity and are found to be good sources of antioxidant. These findings confirm the antioxidant potential of Tunisian date cultivars and increase focus on the impact on health promoting antioxidative compounds in those cultivars during the three maturation stages.

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Notes

The authors declare no competing financial interest.

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