Some Quality Attributes and Biochemical Properties of Nine Iranian Date (*Phoenix dactylifera* L.) Cultivars at Different Stages of Fruit Development

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Abstract
The fruit of date palm (*Phoenix dactylifera* L.) is an important horticultural product in the Middle East and North Africa. Among more than 400 reported date palm cultivars in Iran, around 20 cultivars are more important due to having better eating quality and trading values. In this study, the fruit of nine commercially important date cultivars including ‘Barhee’, ‘Breim’, ‘Deiri’, ‘Fersi’, ‘Gantar’, ‘Khadravi’, ‘Sayer’, ‘Shakar’ and ‘Zahidi’ were evaluated for certain quality and biochemical properties during the last three stages of ripening (i.e., Khalal, Rutab and Tamar). The results showed that the highest levels of total soluble solids (TSS) and titratable acidity were recorded at Tamar stage for all studied cultivars. Maximum TSS was recorded for ‘Deiri’ (77 %) and ‘Zahidi’ (75.3%) which are considered as dry dates. By a similar pattern, the mean amount of soluble proteins, flesh darkening and peroxidase activity (POX) increased to 5587 µg g⁻¹, 0.76 A410 and 5220 Ug⁻¹ tissue, respectively. Fruit at Khalal stage showed an incredible amount of phenolic compounds and antioxidant capacity, but these parameters of nutritional value reduced to their lowest level in almost all studied cultivars at Tamar stage. Flesh darkening as an index of appearance quality increased greatly by turning Rutab into Tamar for almost all cultivars.

Keywords: Date palm, nutritional value, fruit development, Iranian dates.

Introduction
Date fruit (*Phoenix dactylifera* L.) is one of the oldest known fruit crops and is considered as an important component of the diet in many Middle Eastern and North African countries (Chao and Krueger, 2007). The fruit of date is nutritious, which is incredibly rich in carbohydrates, minerals, dietary fibers and amino acids (Al-Shahib and Marshall, 2003). Development and ripening of date fruit is characterized by five different stages known as Hababouk, Kimri, Khalal, Rutab and Tamar, respectively (Fig. 1).

Hababouk stage starts after the fruit set and lasts for 4-5 weeks. During Kimri stage, the fruit is green in color, quite hard, and usually unsuitable for eating. At Khalal stage, fruit color changes to yellow, amber, or red and in some cultivars such as ‘Barhee’ and ‘Breim’, the fruit loses its astringency and becomes sweet and consumable. At Rutab stage, the fruit with
firm texture turns into a soft, sweet and delicious produce in almost all cultivars. At the last maturation stage, i.e., Tamar, fruit water content decreases to less than 24% and flesh texture becomes dry and its color changes to brown dark color (Baliga et al., 2011, Mortazavi et al., 2010). In general, most date cultivars are harvested and marketed in one or more of three last maturation stages i.e. Khalal, Rutab or Tamar depending on market demand and eating quality (Awad et al., 2011). The overall appearance and physicochemical properties of the fruit depend on growth conditions (soil, climate, etc.) and genetic differences. Discovering the physicochemical characteristics of fruit during development is considered as a key factor for better pre- and post-harvest management in different date cultivars. These data could be successfully utilized for product development, quality control, process equipment design, shelf life prediction, packaging, and storage (Hasnaoui et al., 2010; Amira et al., 2011; Hasnaoui, et al., 2011). More than 3,000 varieties of date palm have been reported from all around the world, among them only a few cultivars are concerned with the economic value in each country (Zaid, 1999). In this work the fruit biochemical properties related to quality and nutritional value of nine most common and commercially important Iranian date cultivars were studied during maturation stages.

Materials and Methods

**Plant material**

Fresh fruit samples were harvested from the Iranian Date Palm germplasm collection (48°40’E, 31°19’N) where more than fifty known and commercial date palm varieties are collected from different producing regions. Nine cultivars including ‘Barhee’, ‘Sayer’, ‘Zahidi’, ‘Breim’, ‘Deiri’, ‘Fersi’, ‘Gantar’, ‘Khadravi’ and ‘Shakar’ were selected for this experiment. Fruit samples of three maturation stages, i.e., Khalal, Rutab, and Tamar were harvested and transferred to the lab immediately. For dry dates with no distinctive stage of Rutab (such as ‘Deiri’ and ‘Zahidi’), the fruits which lost their Khalal properties partly were considered as Rutab stage (18-20% water content). Uniform fruits were selected based on indices of the maturity stage and cultivar. The fruits were pitted and edible portions (fruit pulp) were wrapped in the aluminum foil and stored at -80°C until analysis (Lado et al., 2014).

**Total soluble solids, titratable acidity and darkening**

Five grams of fruit pulp were extracted with 30 ml of distilled water using a mortar and pestle. Extract was centrifuged at 6000 rpm, 4°C for 15 min and the supernatant was used for analysis. TSS was evaluated with a digital Atago refractometer and the results were presented as % Brix. To estimate the TA, fruit extract was titrated with 0.01 N NaOH to the end-point of pH 8.1 and results were presented as mg equivalents of malic acid per 100 g FW. For measurement of fruit flesh darkening, the absorbance of fruit extract was read at 410 nm (Baloch et al., 2006).

**Antioxidant capacity and total phenolics**

One gram of fruit homogenate was put in a falcon tube and 10 ml of 80% methanol was
added to it. After shaking vigorously (for 2 h), the mixture was cold centrifuged at 6000 rpm for 20 min and supernatant was used for analysis. The antioxidant capacity was measured using FRAP assay with slight modifications (Benzie and Strain, 1996). Briefly, 80 µl of supernatant was mixed with 3.6 ml FRAP prepared reagent (0.3 M of acetate buffer at pH 3.6, 10 mM 2,4,6-tripyridyltriazine in 40 mM HCl and 20 mM ferric chloride and distilled water at a volume ratio of 10:1:1) and then 400 µl distilled water was added to the mixture. After incubation for 50 min at 37°C, absorbance at 593 nm was measured spectrophotometrically against a FRAP blank. Aqueous solutions of known Fe (II) concentrations (FeSO₄·7H₂O) were used for calibration. Results were expressed as mmol Fe²⁺ equivalents per g sample. To determine the content of total phenolics, 200 µl of supernatant was mixed with 1.5 ml of Folin-Ciocalteu’s reagent (10% v/v). After shaking for 5 minutes, 1.5 ml of sodium carbonate solution was added and the mixture was homogenized. The reaction mixture was incubated in the dark at room temperature for 90 min and then its absorbance was measured at 725 nm. Gallic acid was used as standard and the results expressed as mg gallic acid equivalents (GAE) per 100 g sample (Slinkard and Singleton, 1977).

**Proline**
The modified method of Bates et al. (1973) was used for proline determination. Briefly, 0.5 g of homogenized fruit sample was extracted in 10 ml of sulfosalisilic acid 3%. The mixture was centrifuged at 6000 rpm, 4°C for 20 min. A 1:1:1 mixture of the extract, ninhydrin acid and glacial acetic acid was incubated at 100°C for one hour. The reaction was stopped in an iced bath and vortexed for 30s after adding toluene to the mixture. The obtained pink color in the toluene phase was monitored at 520 nm using a spectrophotometer. The method was calibrated with 0-20 mg L⁻¹ standard proline solutions and results were expressed as mg g⁻¹ fresh weight basis.

**Soluble proteins and peroxidase activity**
Based on the method used by Guy et al. (1992), protein was extracted by homogenizing 2 g of fruit pulp with 10 ml extraction buffer (50 ml Tris-HCL 0.05 M, 2 ml Na-EDTA 0.1 M and 40 µl 2-Mercaptoethanol). Extract was centrifuged at 4°C, 6000 rpm for 20 min and supernatant was used for soluble proteins and peroxidase activity analysis.

**Protein Assay**
The soluble protein content of extracts was determined by the Bradford method (Bradford, 1976). The Bradford reagent was prepared by dissolving 0.1 gram of coomassie brilliant blue G-250 in 50 ml ethanol. After adding 100 ml phosphoric acid (85%), the solution volume was adjusted to 1 L by adding distilled water. From this solution, 2.9 ml was mixed in a tube with 100 µl of fruit extract or standard protein (BSA). After being covered, the tubes were inverted several times and the absorbance of mixture was recorded at 595 nm after 5 min incubation in a dark and cold place. A BSA standard (0 to 1.0 mg mL⁻¹) was prepared from the dilution of a 2 mg/ml stock solution. A blank was prepared by using 100 µl of extraction buffer. Data were released as µg protein per g tissue.

**POX Assay**
The reaction mixture contained 80 ml potassium phosphate buffer (0.1 M, pH 6.5), 10 ml of guaiacol (1%) and 10 ml of hydrogen peroxide (0.3%). For enzyme activity, 0.1 ml of enzyme was added to 2.9 ml of reaction mixture in a cuvette and color development at 470 nm was monitored for 15 min at room temperature (Awad et al., 2011).

**Pigment content determination**
Total chlorophylls, carotenoids and anthocyanins were determined in the exocarp.
layer of fruit at Khalal stage. For chlorophyll, one gram of flesh was extracted with 5ml of 80% cold acetone using mortar and pestle. The extract was centrifuged and then the supernatant absorbance was recorded at 663 and 645 nm (Arnon, 1949). For carotenoids extraction, two grams of tissue were placed in a falcon tube and after adding 18 ml of n-hexane, acetone and ethanol (at the ratio of 1:1:2), it was shaken for 20 min. The mixture was centrifuged at 5000 rpm for 15 min. The absorbance of the hexane layer was read at 450 nm and the concentration of carotenes was calculated by comparison against a calibration curve prepared with the 0-24 µg mL⁻¹ standard solutions of β-carotene (Barry and van Wyk, 2006). The total anthocyanin content was measured using a modified pH differential method (Lee et al., 2005). The absorbance of the methanolic extract of anthocyanins was measured at 510 and 700 nm in different pH buffers (pH 1.0 and 4.5 respectively). Absorbance readings were converted to total mg of cyanidin-3-glucoside per 100 g fresh weight using the molar extinction coefficient of 26,900.

**Statistical design and analysis**

The experiment was set up in a completely randomized design with 9 (cultivars) × 3 (ripening stages) factors with three replicates (totally 27 treatments). Values were expressed as means ± standard errors. Statistical evaluation was performed using MSTATC software (Michigan State University, 1988). Differences among means were compared by the LSD test at 0.05 probability levels.

**Results**

A comparison between the three growth stages in cultivars showed that significantly higher amounts of TA existed at Tamar stage (Fig. 2A). Except for the dry dates which don’t have a distinctive Rutab stage (i.e. ‘Deiri’ and ‘Zahidi’), to some extent reduction in titratable acidity was recorded when fruit turned from Khalal into Rutab and for most cultivars, the lowest level of acidity was recorded at Rutab stage. Maximum and minimum titratable acidity levels (160 and 18 mg/100g FW) were obtained for cv. ‘Zahidi’ at Tamar stage and cv. ‘Shakar’ at Rutab stage, respectively.

Figure 2B shows that TSS increased during fruit development in all cultivars and mean TSS values for Khalal, Rutab, and Tamar stages were 36.2, 56.7, and 70.5%, respectively. Some cultivars which are classified as dry dates, such as ‘Deiri’ and ‘Zahidi’ presented the highest TSS values at both Rutab and Tamar stages. Results showed that among cultivars with edible Khalal, cv. ‘Barhee’ had the highest TSS value (40.13%).

![Fig. 2. Titratable acidity (A) and TSS (B) of some Iranian date palm cultivars at different fruit developmental stages. Bars on the columns represent the ± S.E](image-url)
As shown in Figure 3A, for most cultivars, the level of antioxidant capacity was remarkable at Khalal stage and the exceptions ( cvs. ‘Barhee’, ‘Breim’ and to some extent cv. ‘Sayer’) were the cultivars which had edible Khalal fruit. The lowest level of antioxidant capacity was observed for cv. ‘Barhee’ at Khalal stage (0.71 mol Fe II/100g FW). On comparing, the capacity of antioxidants at Tamar stage was a little more than Rutab stage and cvs. ‘Sayer’ and ‘Khadravi’ had the highest level of antioxidant capacity (1.18 and 1.11 mol Fe II/100g FW, respectively) at the time of commercial harvest.

**Fig. 3.** Antioxidant capacity (A) and total phenols (B) of some Iranian date cultivars at different fruit developmental stages. Bars on the columns represent the ± S.E

The pattern of changes for the amount of total phenolics was almost identical to what was obtained for antioxidant capacity (Fig. 3 B). Except for cv. ‘Barhee’, other cultivars showed the most amount of TP content at Khalal stage, and then fruit at Tamar stage had a slightly more amount of TP than Rutab fruit. The highest and lowest values obtained for TP were in Khalal stage of ‘Deiri’ (2624.8 mg GA/Kg FW) and Rutab stage of cv. ‘Shakar’ (230.3 mg GA/Kg FW), respectively.

The amount of proline as a key amino acid in plant materials showed an increasing pattern during fruit development (Fig. 4 A). Mean proline content for three developmental stages, i.e., Khalal, Rutab, and Tamar were 27.6, 68.7, and 105.2 mg/g FW, respectively. Maximum proline value was detected for the fruit of cv. ‘Breim’ at Tamar stage (189.2 mg g⁻¹ FW).

Soluble protein content was determined at Rutab and Tamar stages and obtained data showed a sharp increase in protein content at Tamar stage (Fig. 4B). Maximum protein content was recorded in Tamar fruit of some cultivars including ‘Gantar’, ‘Deiri’ and ‘Shakar’ (7693.5, 7604.6 and 7240.7 µg g⁻¹ FW, respectively). Also, the lowest level of soluble protein content was observed in Rutab stage of cv. ‘Zahidi’.

Flesh darkening is considered as an acceptable index of date color. As expected, in most cultivars, fruits at Tamar stage had more darkening index and around two-fold increase in darkening index was recorded for some cultivars such as ‘Khadravi’ and ‘Gantar’ when fruit turned into Tamar (Fig. 5A). In contrast, in some cultivars (i.e., ‘Barhee’ and ‘Fersi’) there were no significant differences between the two stages of Rutab and Tamar. Maximum darkening values were recorded for cvs. ‘Khadravi’, ‘Sayer’ and ‘Gantar’ at Tamar stage (1.18, 0.87 and 0.79 Abs at 410 nm, respectively).

As illustrated in Figure 5B, great differences were obtained for POX activity of different date cultivars and between...
Rutab and Tamar developmental stages. No activity was recorded for Tamar stage of cvs. ‘Deiri’, ‘Zahidi’ and ‘Fersi’, but on the contrary, high levels of peroxidase activity were seen in Tamar fruit of some other cultivars, i.e., ‘Breim’ and ‘Shakar’ (5216.4 and 2880.9 U g⁻¹ FW).

Pigment analysis of date fruit was done only at Khalal stage and the data of Table 1 demonstrate that cv. ‘Deiri’ had the highest level of chlorophyll content (22.7 µg g⁻¹ tissue). Minimum chlorophyll values were recorded for cvs. ‘Fersi’ and ‘Gantar’ (7.7 and 9.3 µg g⁻¹, respectively). There were no significant differences between other cultivars in chlorophyll content. Maximum levels of carotenoids were seen in ‘Sayer’, ‘Khadravi’, and ‘Barhee’ cultivars (>23 µg g⁻¹ tissue). Some cultivars such as ‘Zahidi’, ‘Fersi’, and ‘Gantar’ had the mean values and the lowest carotenoid content was recorded for cv. ‘Breim’ (8.5 µg g⁻¹ tissue). Data on total anthocyanins revealed that fruit at Khalal stage is very poor in terms of having this pigment. Maximum anthocyanin content was observed in cvs. ‘Deiri’, ‘Breim’, and ‘Barhee’ (> 3 mg 100g⁻¹) which are negligible compared to other horticultural products.

Fig. 4. The amount of proline content (A) and total protein (B) of common Iranian date palm cultivars at different fruit developmental stages. Bars on the columns represent the ± S.E

Fig. 5. Changes in fruit darkening (A) and peroxidase activity (B) of common Iranian date palm cultivars at different fruit developmental stages. Bars on the columns represent the ± S.E
Table 1. Total chlorophylls, carotenoids and anthocyanins of nine Iranian date fruits at Khalal stage (Mean ±S.E.)

<table>
<thead>
<tr>
<th>Cultivar</th>
<th>Total chlorophyll (µg g⁻¹)</th>
<th>Total carotenoid (µg g⁻¹)</th>
<th>Total anthocyanin (mg 100 g⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>‘Sayer’</td>
<td>13.08±1.96</td>
<td>24.31±0.40</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>‘Barhee’</td>
<td>14.82±2.27</td>
<td>23.52±0.20</td>
<td>3.10±0.29</td>
</tr>
<tr>
<td>‘Breim’</td>
<td>10.08±0.35</td>
<td>8.48±0.48</td>
<td>3.71±0.62</td>
</tr>
<tr>
<td>‘Khadravi’</td>
<td>11.54±1.55</td>
<td>23.94±0.42</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>‘Deiri’</td>
<td>22.77±2.09</td>
<td>17.75±1.80</td>
<td>3.71±0.13</td>
</tr>
<tr>
<td>‘Zahidi’</td>
<td>11.22±1.26</td>
<td>19.06±0.38</td>
<td>0.00±0.00</td>
</tr>
<tr>
<td>‘Deiri’</td>
<td>22.77±2.09</td>
<td>17.75±1.80</td>
<td>3.71±0.13</td>
</tr>
<tr>
<td>‘Shakar’</td>
<td>12.41±0.31</td>
<td>14.15±0.45</td>
<td>1.64±0.20</td>
</tr>
<tr>
<td>‘Fersi’</td>
<td>7.71±1.53</td>
<td>18.40±1.44</td>
<td>1.14±0.06</td>
</tr>
<tr>
<td>‘Gantar’</td>
<td>9.35±0.75</td>
<td>16.37±1.02</td>
<td>1.68±0.13</td>
</tr>
</tbody>
</table>

Table 2. Correlation analysis between different characteristics of nine Iranian date fruits

<table>
<thead>
<tr>
<th></th>
<th>TSS</th>
<th>TA</th>
<th>AC</th>
<th>TP</th>
<th>Pr</th>
<th>Dr</th>
<th>SP</th>
<th>POX</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSS</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Titratable Acidity (TA)</td>
<td>0.69**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antioxidant Capacity (AC)</td>
<td>-0.57**</td>
<td>-0.20ns</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total Phenols (TP)</td>
<td>-0.34**</td>
<td>-0.01ns</td>
<td>0.84**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Proline (Pr)</td>
<td>0.69**</td>
<td>0.43**</td>
<td>-0.49**</td>
<td>-0.34**</td>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Darkening (Dr)</td>
<td>0.06**</td>
<td>0.00ns</td>
<td>-0.14ns</td>
<td>-0.24ns</td>
<td>0.04**</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Soluble Protein (SP)</td>
<td>0.09**</td>
<td>0.32*</td>
<td>0.02**</td>
<td>-0.12**</td>
<td>-0.17**</td>
<td>0.61**</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>POX</td>
<td>0.19ns</td>
<td>0.07**</td>
<td>-0.24ns</td>
<td>-0.11ns</td>
<td>0.19**</td>
<td>0.13ns</td>
<td>0.27*</td>
<td>1</td>
</tr>
</tbody>
</table>

ns: non-significant; *: significant at P≤0.05; **: significant at P≤0.01

As shown in Table 2, a significant positive correlation was observed between antioxidant capacity and total phenols. In addition, soluble protein content was in correlation with darkening and POX activity. The concentration of proline amino acid was positively correlated with TSS and titratable acidity and negatively correlated with phenolic content and antioxidant capacity.

Discussion

TSS and titratable acidity are the main factors affecting fruit taste. TSS showed an increasing trend during fruit development. High TSS values represent the high percentage of sugars especially in cvs. ‘Deiri’ and ‘Zahidi’ which have lower water content. Fruit sweetening in the final stages of development is seen in most fruits and can be attributed to the hydrolytic conversion of insoluble carbohydrate polymers into low density soluble sugars (Saleem et al., 2005). But in the case of date fruit, the loss of a substantial portion of water enhances concentration of soluble solids. This issue affects both the taste and the texture of date fruit, and makes the fruit much sweeter (Mortazavi et al., 2010). Also among known cultivars with edible fruit at Khalal stage, maximum TSS value was recorded for cv. ‘Barhee’ which makes it the most popular cultivar worldwide as a sweet fresh date (Zaid, 1999).

A consideration of the data presented for titratable acidity shows that date fruit with maximum acidity of around 0.1% can be considered as a low acidity fruit, and it seems that this parameter has a minor effect on fruit taste. Reducing the level of acidity at Rutab stage of most cultivars can be due to the conversion of part of organic acids into sugars, but at the last stage of fruit development, i.e., Tamar, the fruit has
very low metabolic activity, so the increase in the level of titratable acidity can be related to the considerable water loss and juice concentration at this stage.

The same trend of changes for antioxidant capacity and total phenolics strongly suggested the main role of phenolic compounds in fruit antioxidant capacity. Phenolic compounds are widely distributed in plants. Not only do they contribute to color and flavor and are responsible for astringent and bitter tastes in fruits and vegetables, they also have antioxidant properties (Ding et al. 2001). Phenolic compounds consist of simple phenols, benzoic and cinnamic acid, coumarins, tannins, lignins, lignans and flavonoids (Khoddami et al., 2013). Among the three developmental stages, maximum phenolic content was recorded for Khalal stage. The fact that the highest levels of phenolic content exist in the most astringent cultivars, i.e., ‘Deiri’, ‘Zahidi’, and ‘Gantar’ and on the other hand, the lowest recorded phenolic content in the Khalal fruit of cv. ‘Barhee’ which has a sweet taste and low astringency confirms that tannins are the main phenolic compounds of date fruits. Tannins are bitter plant polyphenolic compounds that bind to and precipitate proteins and various other organic compounds including amino acids and alkaloids. The significant reduction of astringency after turning Khalal into Rutab can be attributed to the deposition of hydrolysable tannins in developing fruits (Sriyook et al., 1994).

Proline is a proteinogenic amino acid which is essential for primary metabolism. Most of the research concerning proline metabolism in plants has been focused on its accumulation in vegetative tissues (Stines et al., 1999). Proline accumulation in plant organs has been reported during conditions of drought, high salinity, high light and UV irradiation, heavy metals, oxidative stress and in response to biotic stresses (Szabados and Savoure, 2009). Proline may act as an osmoticum, a sink of energy and reducing power, a nitrogen-storage compound, a hydroxy-radical scavenger, and a compatible solute that protects enzymes (Saradhi and Saradhi, 1991). Increase in the level of proline during fruit development in date cultivars took place when fruits lost considerable portions of their water due to extremely hot weather and dry winds at September. These stressful conditions might be the main reasons for the highest proline concentration at the last stages of date fruit development.

The data obtained for soluble protein content clearly showed the increasing rate of protein concentration at Tamar stage. Al-Shabib and Marshall (2003) reported that date fruits contain a higher percentage of protein than other types of fruits. Twenty-three different amino acids have been found in the proteins of dates, many of which are not present in the most popular fruits such as oranges, apples, and bananas. Also El-Sohaimy and Hafez (2010) announced that the date fruit extract contains suitable amounts of essential amino acids due to the rise in its nutrition value.

Fruit darkening is a common and expected phenomenon during date fruit maturation, and it relates to enzymatic and non-enzymatic reactions which take place during development, handling, and processing. These reactions are one of the most serious alterations of date in storage and play a major role in the marketing of dates locally and internationally (Khali and Selselet-Attou, 2007).

Peroxidases refer to a group of enzymes which have been involved in deteriorative reactions related to flavor, texture, and color in raw and processed fruits and vegetables. The correlation between peroxidase activity and fruit ripening has been documented in a number of cases (Neves, 2002; Pandey, et al., 2012; Mondal et al., 2009). This experiment showed that some date cultivars had more peroxidase activity at Tamar stage. The peroxidase
activity in different kinds of date fruits was also studied by Awad et al. (2011), and they reported that the POX was initially low at Hababouk stage and then sharply increased during fruit development in all cultivars. Higher peroxidase activity can be involved in the oxidation of phenolic compounds and formation of dark brown pigments and affecting fruit color.

Pigment content including chlorophylls, carotenoids, and anthocyanins are responsible for green, yellow, and red colors in Khaled date fruit, respectively (Bacha, et al., 1987; Al-Farsi, 2005). In almost all the studied cultivars, the color green disappeared at Khalal stage and a comparison between chlorophyll content at Khalal stage and what is reported by others for early stages of development (Bacha et al., 1987; Mortazavi, 2007) represents considerable degradation of chlorophylls during fruit development. The concentration and composition of some date fruit carotenoids was studied by different authors who determined their major role in fruit yellow color at Khalal stage (Mortazavi, 2007; Boudries et al., 2007). Anthocyanins existed in minute amounts in the various studied cultivars except in cvs. ‘Deiri’, ‘Breim’ and ‘Barhee’. Both ‘Breim’ and ‘Deiri’ have red to dark red color at Khalal stage and in the case of ‘Barhee’ it seems that anthocyanin color is overcome by high concentration of carotenoids.

**Conclusion**

From the results presented in this work, it can be concluded that there are important biochemical differences between the studied cultivars. Fruit development involved severe biochemical changes. Among the three developmental stages, maximum levels of TSS, titratable acidity, soluble proteins, proline, peroxidase activity, and darkening index were recorded at Tamar stage, and it demonstrates that the fruit of most date cultivars are exposed to marked biochemical changes when turning into Tamar. In addition, some of the studied traits, i.e., antioxidant capacity and total phenolics were at the highest levels at Khalal stage when the fruit of most cultivars are not edible due to high astringency. On the other hand, these valued nutritional parameters drop noticeably at harvest time. More research is needed to clarify the role of enzymes in the changes of date fruit quality and comparing different date cultivars with respect to factors affecting fruit quality.

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