Antioxidant Content and Capacity of Jordanian Date Palm Fruit at two Maturity Stages

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Abstract

Introduction: Date palm (Phoenix dactylifera L.) is cultivated in Jordan for long known time. One of the most commonly grown date palm fruit varieties in Jordan is Barhi. The date palm fruits get matured due to the enzymatic action upon fruit components triggered by climatic conditions. This maturity can be felt through changes in fruit color, size, weight, and taste. Date palm fruit has three edible maturity stages i.e. Khalal, Rutab, and Tamr. Tamr has the least amount of moisture. This study aimed at comparing the antioxidant capacity and the capacity of Jordanian Barhi variety of date palm fruit at two maturity stages such as Rutab and Tamr on fresh matter basis. Methods: Two methods were used to study the antioxidant content namely Folin-Ciocalteau method and total flavonoid method whereas 2,2-diphenyl-1-picrylhydrazyl (DPPH) and cupric antioxidant reducing capacity (CUPRAC) assays were used to study the antioxidant capacity. Three solvents were used for the fruit extraction (ethanol, methanol, and water). Results: From the results, it was inferred that different extracts of Rutab and Tamr showed significant (P<0.05) differences in terms of antioxidant content as well as antioxidant capacity. Rutab showed significantly (P<0.05) high total polyphenol content (measured by Folin-Ciocalteau method as M catechin/100 g) than Tamr which reflected in its high antioxidant capacity (measured as M trolox/100 g by CUPRAC assay) in Rutab. On the other hand, Tamr exhibited higher total flavonoid content (measured as M rutin/100 g by total flavonoid method) than Rutab. This result has been reflected by higher antioxidant capacity (measured as % DPPH scavenging capacity and as vitamin C equivalent by DPPH assay) in Tamr. Conclusion: Upon maturation from Rutab to Tamr, antioxidant content decreases while total flavonoid content increases. The antioxidant capacity increases while the %DPPH scavenging diminishes upon maturation from Rutab to Tamr. Further, some antioxidants diminish while others get concentrated upon maturation from Rutab to Tamr: The use of different solvents for extraction allowed the extraction and quantification of different polarity antioxidants of the fruit.

Key words: Date palm fruit (Phoenix dactylifera L.), Rutab, Tamr, antioxidant content, antioxidant capacity.

INTRODUCTION

Date palm (Phoenix dactylifera L.) is a perennial monocotyledon that belongs to Palmae family (Food and Agricultural Organization, FAO, 1993). Date palm has been cultivated as early as 4000 B.C. (Chao and Knueger, 2007; El-Juhany, 2010) in the Middle Eastern countries (FAO, 1993) though it is considered as a recent habit in Jordan. The most common date palm varieties that are cultivated in Jordan are Haimi, Red Talal and Barhi (Ministry of Agriculture (MOA), 2008). Date palm fruit has been proved as a source of many functional constituents such as antioxidant vitamins, flavonoids, and polyphenols (Al-Farsi and Lee, 2008) and used in medical applications (Al-Shahib and Marshall, 2003).

Several studies examined the antioxidant capacity of various extracts of different date palm fruit varieties (Ahmed et al., 1995; Qusti et al.,2010). According to the available literature, no study compared the antioxidant content and capacity of date palm fruit of Jordanian Barhi variety at two maturity stages i.e., Rutab and Tamr in terms of fresh matter. Thus, this study is aimed at comparing the antioxidant content and capacity of date palm fruit of Jordanian Barhi variety at two maturity stages i.e., Rutab and Tamr on fresh matter basis. Two methods were deployed to study the antioxidant content; namely: Folin-Ciocalteau method and total flavonoid method. Two methods were used to study the antioxidant capacity; namely: 2, 2-diphenyl-1-picrylhydrazyl (DPPH) and cupric antioxidant reducing capacity (CUPRAC) assays. Three solvents (ethanol, methanol, and water) were used for the fruit extraction.

2. Literature review:

The maturity observed in the date palm fruits is due to enzymatic action upon fruit components induced by climatic conditions. This can be observed through the changes in the fruit color, size, weight, and taste (Allaith, 2008; Biglari et al., 2008). Before consumption, the fruit should pass through the maturity stages such as Hababouk and Kimeri. Then, the fruit passes through some chemical changes related to water and sugar content in order to be edible at three different maturity stages: Khalal, Rutab, and Tamr (Lobo et al., 2013). To reach the Khalal stage, slow paced processes such as weight gain of the fruit, increase in sucrose content, decrease in moisture content, and tannin precipitation occurs which finally makes the fruit edible (Bacha, 1987, FAO, 1993). Upon ripening from Khalal to Rutab, the water content of the fruit gets reduced, and during Tamr stage, the water is further reduced and accordingly, the fruit becomes self-preserved (Bacha et al., 1987; FAO, 1993; Ahmed et al., 1995).

2. Methodology:

The fruits at the maturity stages Rutab and Tamr were purchased from local market and analyzed. As reported by the seller, the fruit at the Rutab stage was collected in the same day of purchasing (morning), neither stored nor treated. The fruit at the maturity stage Rutab was prepared by washing with tap water and gentle drying by towel paper. Samples were then cut finely by knife or food chopper (Arteco, China). About 1-3 g representative samples were conventionally...
extracted by 10 ml of the one of three extraction solvents (water, methanol, ethanol) at 90°C, 50°C, and 50°C respectively for 2 hours with intermittent shaking. The extracts were then centrifuged at 3000 rpm for 10-15 minutes (Himax®, Germany) and filtered (Whatman filter paper No.4), purged with liquid nitrogen, and stored at -20°C (for not more than two months) until analyzed. Deionized water was used for the preparation of all standard solutions and to complete the reactions (Apak et al., 2007).

2.A. Determination of antioxidant content:
Chemicals were purchased from GCC® (UK), Fischer® (China), Labscan® (Thailand), LabChem® (USA) and Sigma® (China). Standard curves were prepared to have r² value of 0.96-0.99. Samples were analyzed in duplicate with an accuracy of not less than 95% (Laterotti et al., 2006) and coefficient of variation of not more than 15%. Absorbance values were measured using UV-visible spectrophotometer (Sco Tech, Model SPUV®) at the specified wavelength values against standard concentrations of certain antioxidants and blank solutions.

2.A.1. Folin-Ciocalteau method:
Folin-Ciocalteau method was used for the determination of antioxidant content according to Agbor et al. (2014). Sample volume (10-100 μl) was completed to 1000 μl by 10x freshly prepared Folfin-Ciocalteau reagent to complete the reaction within 15- minutes. Sample concentration for antioxidants was measured against freshly prepared catechin standard (catechin standard was dissolved in methanol) at 750 nm wavelength.

2.A.2. Total flavonoid method:
Total flavonoids were analyzed as described previously (Pękal and Pyrzynka, 2014). One milliliter sample was added to methanolic solution (2% w/v) of AlCl₃(0.5 ml). Then, 0.5 ml of deionized water and 0.5 ml of 1M HCl were added respectively, the mixture was shaken vigorously to complete the reaction within 10 minutes. The absorbance was measured at 415 nm wavelength against different concentrations of rutin standard solutions (rutin was dissolved either in ethanol or in methanol).

2.B. Determination of antioxidant capacity:
2.B.1. CUPRAC Assay:
CUPRAC assay was performed as described previously by Apak et al., (2007). To a suitable amount of sample (0.5-5 ml), 10.21 ml concentrated (36%) HCl was added, reaction volume was then completed to 100 ml by 50% methanol, refluxed at 80°C for 2 hours, and cooled down to room temperature. Sample mixture was then neutralized to pH 7 by 1M NaOH. Then, 1 ml CuCl₂, 1 ml neocuprine, 1 ml acetate buffer, and suitable sample volume (500-1100 μl) were added respectively to complete the reaction volume to 4.1 ml. The reaction mixture was then incubated at 50°C for 20 minutes, cooled to room temperature and centrifuged at 3000 rpm for about 7 minutes. Sample absorbance was measured using a spectrophotometer at 450 nm (Apak et al., 2007) against different concentrations of trolox standard solutions (trolox was dissolved either in ethanol or in methanol).

2.B.2. DPPH assay:
The DPPH assay procedure was performed according to Molyneux (2003). The free radical 2,2-diphenyl-1-picrylhydrazyl (DPPH) (2.95 ml of 0.1 mM, prepared in 80% ethanol) was added to 50 μl sample. The mixture was incubated at room temperature for 30 minutes in dark place. The absorbance was then measured at 517 nm wavelength against ascorbic acid as a standard. The scavenging percentage was calculated according to the following equation:

Scavenging effect (%) = \( \frac{A_0 - A_1}{A_0} \times 100\% \)

Where:
A₀ is the absorbance of the control
A₁ is the absorbance of the sample

2.C. Statistical analysis
The statistical analysis of data was performed using the software package for social sciences (SPSS, version 23). To detect the differences between the 2maturity stages of the fruitand the solvent of extraction, data were analyzed by factorial mixed (effect of type of fruit and extraction solvent) analysis of variance (ANOVA) (Laerd, 2018). Significant differences were considered at P<0.05. Data are expressed in the tables as mean ± standard deviation.

3Results:
Table 1 shows the antioxidant content (Mrutin/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (Phoenix dactylifera L.) at the two maturity stages Rutab and Tamr determined by Folin-Ciocalteau method. The fruit at the Rutab stage contained higher (P<0.001) concentration of antioxidants. Ethanol and (P>0.05) water extracted antioxidants more (P<0.001) than methanol did from Rutab. On the other hand, water followed (P<0.05) by methanol extracted antioxidants more (P<0.05) than ethanol did from Tamr.

Table 1: The antioxidant content (Mrutin/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (Phoenix dactylifera L.) at the two maturity stages Rutab and Tamrdetermined by Folin-Ciocalteau method.

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Antioxidant content (Mrutin/100g) as determined by Folin-Ciocalteau method</th>
<th>Extract</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutab</td>
<td>ethanol 4.9030±0.3766 methanol 2.3459±0.0357 water 3.7452±1.1798</td>
<td></td>
<td>0.000 ²</td>
</tr>
<tr>
<td>Tamr</td>
<td>0.6269±0.0541 1.7263±0.0698 2.7589±0.1096</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

²Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%
³P values are used to express significant differences between Rutab and Tamr extracts at P<0.05.

Table 2: The antioxidant content (Mcatechin/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (Phoenix dactylifera L.) at the two maturity stages Rutab and Tamrdetermined by total flavonoid method.

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Antioxidant content (Mcatechin/100g) as determined by total flavonoid method</th>
<th>Extract</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutab</td>
<td>ethanol 32.2065±1.4925 methanol 9.0094±0.2760 water 9.0607±0.1085</td>
<td></td>
<td>0.000 ³</td>
</tr>
</tbody>
</table>

³P values are used to express significant differences between Rutab and Tamr extracts at P<0.05.

Discussion:
The results for total polyphenols retrieved in this research (determined by Folin-Ciocalteu method) are in alignment with that of El Sohaimy et al. (2015) who found 1.10 µg cateching Tamr in Egyptian dates (through HPLC analysis). The values (i.e., the average of the three extracts) obtained in this research correspond to 1.06 µg cateching Rutab and 1.81 µg cateching Tamr. On the other hand, Saleh et al. (2011) found values much higher than the current study values (7.3, 7.5 and 5 µg/kg Tamrod Ajwa, Sukkari, and Khalas varieties).

Table 3: The antioxidant activity (M trolox/100g) of the methanolic, ethanolic, and water extracts of date palm fruit (Phoenix dactylifera L.) at Rutab and Tamr determined by CUPRAC assay.1,3,4

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Antioxidant activity (M trolox/100g) as determined by total CUPRAC assay</th>
<th>Extract</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Water</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutab</td>
<td>1.5005±0.0647</td>
<td>4.9531±0.0989</td>
<td>1.9141±0.0301</td>
<td>7.1669±0.1856</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamr</td>
<td>1.5612±0.0080</td>
<td>4.3823±0.0793</td>
<td>1.9141±0.0301</td>
<td>7.1669±0.1856</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%

P values are used to express significant differences between Rutab and Tamrextracts at P<0.05.

Table 4: The antioxidant activity (expressed as % of DPPH radical scavenging) of the methanolic, ethanolic, and water extracts of date palm fruit (Phoenix dactylifera L.) at Rutab and Tamr maturity stages determined by DPPH assay1,3,4

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Antioxidant activity (expressed as % of DPPH radical scavenging) as determined by total DPPH assay</th>
<th>Extract</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Water</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rutab</td>
<td>35.2654±2.5656</td>
<td>35.8998±1.6359</td>
<td>45.3208±0.8192</td>
<td>50.0000±1.0083</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tamr</td>
<td>11.4602±0.0626</td>
<td>31.5130±0.5064</td>
<td>45.3208±0.8192</td>
<td>50.0000±1.0083</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%

P values are used to express significant differences between Rutab and Tamrextracts at P<0.05.

Table 5: The antioxidant activity (expressed as g/ml vitamin C equivalent) of the methanolic, ethanolic, and water extracts of date palm fruit (Phoenix dactylifera L.) at Rutab and Tamr maturity stages determined by DPPH assay1,3,4

<table>
<thead>
<tr>
<th>Maturity stage</th>
<th>Antioxidant activity (expressed as g/ml vitamin C equivalent) as determined by total DPPH assay</th>
<th>Extract</th>
<th>Ethanol</th>
<th>Methanol</th>
<th>Water</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tamr</td>
<td>75.5949±0.4127</td>
<td>402.4646±6.4678</td>
<td>421.9409±8.5093</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Values of the tables are average of duplicates ± SEM with c.v. of not more than 15%

P values are used to express significant differences between Rutab and Tamrextracts at P<0.05.
The figures of total flavonoids found in this research for *Rutabara* are in alignment with that of *Tamr* investigated by Saleh et al. (2011). These researchers found 6.5, 8.10, and 3.60 mg rutin/kg *Tamr* in *Ajwa*, *Sukkari* and *Khalas* varieties respectively. The values found in this research correspond to 10.2 and 35.7 mg rutin/kg *Rutab* and *Tamr* (i.e., the average of the three extraction solvents) respectively.

The CUPRAC assay results are in alignment with that of the values found in other reports (Lemine et al., 2014; Gököç, 2016) who obtained much higher values (75.6 - 99.3 μmol trolox equivalent in six different varieties as 1.40 μmol/g Turkish *Tamr*). Our values correspond to 18.33 μmol trolox activity equivalent/100 g *Rutab* and 9.08 μmol trolox activity equivalent/100 g *Tamr*. These differences are quite natural since the current study used CUPRAC assay whereas the other researchers used DPPH assay. The most important point to be noted is that the study by Lemini et al. (2014) found a percentage loss of antioxidant activity (measured as trolox equivalent) by 0.4-39.1% upon maturation from *Khalal* stage to *Tamr* stage (range is due to different varieties). In this research, the percentage of difference of antioxidant activity between *Rutab* and *Tamr* (upon ethanol extraction only) is 50.4%. DPPH values reported in other studies (72.915%, average of two extracts) were double the values obtained in this study (30% for the *Tamr* stage, average of the three solvents) (El Sohaimy et al., 2015). The critical information to be noted here is that similar to the current study results, water was found to be more powerful in antioxidant extraction than ethanol (El Sohaimy et al., 2015). In accordance to the DPPH% results tabulated in table 4, the current study results for antioxidant capacity (expressed as vitamin C equivalent, Table 5) are much higher than the values reported in literature (Saleh et al., 2011; Lemine et al., 2014; El Sohaimy et al., 2015).

The differences between the current study results and the results of other reports might be due to the difference between the maturity stages analyzed, methods of analysis, experimental standardization conditions, variety (Biglari et al., 2008; Qusti et al., 2010) studied and geographical area where the samples were collected (Halvorsen et al., 2002; Boundries et al., 2007; Qusti et al., 2010; Saleh et al., 2011).

The increased antioxidant content (measured by total flavonoid method, Table 2) upon maturation from *Rutab* to *Tamr* might be due to the reduction in moisture content that might have led to the concentration of antioxidants. This got reflected in the increase in antioxidant capacity (measured by CUPRAC assay, Table 3). Similar to the current study results, at *Tamr* stage, Qusti et al. (2010) found a significant $r^2=0.605$, $P<0.05$ correlation between the antioxidant activity (assayed by ICSO) and phenolic content (mg GAE/g edible fruit).

The reduction in antioxidant content (measured by Folin-Ciocalteau method, Table 1) upon maturation from *Rutab* to *Tamr* probably reflects some decomposition of antioxidants which might have occurred either due to heat or storage (Thompson et al., 2006; Kuhne et al., 2009). This proposed decomposition was reflected by the reduction in antioxidant capacity (measured by DPPH assay, Tables 4 and 5). Similar to the current study results, Aliath (2008) analyzed the relationship among the content of different functional components, characteristics of the date palm fruit and the antioxidant activity (evaluated by FRAP assay). Upon maturation from *Khalal* to *Rutab*, he found a significant negative correlation ($r = -0.267$, $P<0.01$) between the antioxidant activity and ripening. At the *Rutab* stage, a significant negative correlation was found between the antioxidant activity and color ($r = -0.318$, $P<0.01$). These results are in alignment with that of the study by Biglari et al. (2008) who reported in literature that the study by El Sohaimy et al. (2015) for antioxidant content and capacity. It is more powerful in antioxidant extraction than ethanol (El Sohaimy et al., 2015). In accordance to the DPPH% results tabulated in table 4, the current study results for antioxidant capacity (expressed as vitamin C equivalent, Table 5) are much higher than the values reported in literature (Saleh et al., 2011; Lemine et al., 2014; El Sohaimy et al., 2015).

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Using different solvents of different polarities in this research allowed the extraction of different antioxidants thus providing a scientific value to the results found in this investigation. A close review of literature regarding the bioactive components of the date palm fruit shows that the fruit contains phenols (Qusti et al., 2008; Chira et al., 2007), carotenoids such as lycopene, violaxanthin, leukoxanthin (Vayalil, 2012), α and β-carotenes, zeaxanthin, neoxanthin and lutein (Al-Farsi and Lee, 2010; Boundries et al., 2007; United States Department of Agriculture (USDA), 2010). Furthermore, the fruit contains polyphenols (Saleh et al., 2011) flavonoids (with their different classes i.e. flavones such as luteolin and apigenein, flavonols such as quercetin and isorhamnetin, flavonones) (Boudries et al., 2007; Vayalil, 2012) anthocyanins (Chira et al., 2007; Al-Farsi and Lee, 2008), phenolic acids such as p-hydroxybenzoic, syringic, vanillic, caffeic, p-coumaric, ferulic (Allaith, 2008), snipic acids (El-Rayes, 2009), protocatechuic, p-hydroxybenzoic, chlorogenic, isochlorogenic, and datyleric acid (Vayalil, 2012), metals such as Se, Cu, Zn, and Mn, enzymes such as: phtase, invertase, peroxidase (Qusti et al., 2010) and antioxidant vitamins such as the vitamins C and E (Saafi et al., 2011). The presence of such diverse compounds in the date palm fruit make it capable of possessing antioxidant capacity as shown in tables 3, 4, and 5.

The date palm fruit at the *Tamr* stage is assumed to be moderate in its antioxidant content and activity (Qusti et al., 2010) and similar to those present in lemon and sweet cherry (Halvorsen et al., 2002) in terms of amounts, but higher than those found in tomatoes (Zujko and Witkowska, 2011), mango (Pellegrini et al., 2006), spinach, garlic, broccoli, kiwi fruit, figs (Halvorsen et al., 2006), radish, carrots, potatoes, fennel, cabbage, oats, and rice (Zujko and Witkowska, 2011). The date palm fruit at the *Rutab* and *Tamr* stages contain high amounts of free and total phenols (on fresh weight basis) than those in apricots, cranberry, figs, green grapes, and plums (Vinson et al., 2005). This characteristic was proved during in vitro (Allaith, 2008; Khanavi et al., 2009; Qusti, 2010; Saleh et al., 2011) and in vivo investigations (Abo-El-Souad et al., 2004; Khanavi et al., 2009; Saafi et al., 2011; Vayalil, 2012).

### CONCLUSION

Date palm fruit (*Phoenix dactylifera* L.) at *Rutab* and *Tamr* maturity stages contains different types of antioxidants and exhibits antioxidant capacity. Upon maturation from *Rutab* to *Tamr*, the antioxidant content and the capacity of the fruit change.

5. **Limitations and recommendations:**

   a. Limitations of this research include the analysis of only two maturity stages and only one variety of the fruit for antioxidant content and capacity. It is recommended -thus- to analyze different varieties of the fruit and include the edible maturity stage of *Khalal* in the analysis. The use of experimental methods that identify the antioxidant compounds of the fruit at the three edible maturity stages is recommended to add greater value for scientific antioxidant database.

6. **Funding institution:**

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7. **Author contribution:**

   Dr. Hiba Al-Sayyed and Miss Salma AbdelQader did the experimental procedures, statistical analysis of data, and wrote the manuscript. Dr. Refat Al-Kurd and Prof. Marwan Mwalla revised the manuscript.

8. **Conflict of interest:**

   The authors declare that there is no conflict of interest among them.

### REFERENCES


Gökçen, İ., 2016. Total phenolics, antioxidant capacity, colour and drying characteristics of date fruit dried with different methods. Food Science and Technology, ISSN 0101-2061.


