

## Identifying the Natural Antioxidants and Total Phenols of some Date Varieties in Saudi Arabia

**Olfat M. Nassar<sup>1</sup>, Mohamed A. Amin<sup>2</sup>,  
Mohamed E. Hassan<sup>3,4\*</sup> and Heba Ali Nasr<sup>5</sup>**

<sup>1</sup>Department of Home Economics (Nutrition), Faculty of Science and Arts,  
Northern Border University (Rafha), Saudi Arabia.

<sup>2</sup>Department of Botany and Microbiology, Faculty of Science, Al-Azhar University, Cairo, Egypt.

<sup>3</sup>Center of Excellence, advanced material & Nanobiotechnology Group,  
National Research Centre, 12622 Dokki, Giza, Egypt.

<sup>4</sup>Chemistry of Natural and Microbial Products Department, National Research Centre,  
12622 Dokki, Giza, Egypt.

<sup>5</sup>Department of Biology, Faculty of Science and Arts,  
Northern Border University (Rafha), Saudi Arabia.

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***Phoenix dactylifera L.* (date palm) is well known for its innumerable health benefits and nutritional values. Therefore, the present work aimed to determine the total phenolic content in five date palm fruit varieties, Barhi, Barni Al Madina, Khesab, Sukkary and Ajwa, collected from different regions of Saudi Arabia, extracted with aqueous method and to evaluate in vitro their antioxidative properties by DPPH\* method. Consequently, the total polyphenol contents of these extracts will be measured using Folin Ciocalteu spectrophotometric method. Total phenolic content ranged from 57.8 and 55.2 mg of gallic acid equivalent /30 g fresh wt. and the antioxidant activity was ranging from a low value of 29.2 in Sukkary cultivar to a high value of 31.8 in Barni Al Madina cultivar. Also the best carrier for encapsulation of date Palm extracts is CMC comparing with alginate and carrageenan.**

**Key words:** *Phoenix dactylifera*, Encapsulation, total phenolic content, antioxidant activity.

The role of traditional medicines in the solution of health problems is invaluable on a global level. Medicinal plants continue to provide valuable therapeutic agents, both in modern and in traditional medicine<sup>1</sup>.

Date palm is native to North Africa and Persian Gulf regions but its exact origin is uncertain. Top ten producer of *Phoenix dactylifera* are Iraq, Egypt, Saudi Arabia, Tunisia, Algeria, UAE, Oman, Libya Arab Jamahiriya, Pakistan, Sudan, Europe, and USA<sup>2</sup>.

The date palm (*Phoenix dactylifera L.*) is one of oldest cultivated plants of human kind and

used as food for 6000 years<sup>3</sup>. There are more than two hundred varieties<sup>4</sup> of dates available worldwide. It is the main crop in Egypt, Saudi Arabia, and Middle Eastern countries. Each type of dates has shown medicinal value in various type of disease prevention. Dates and their constituents show a role in diseases prevention through antioxidant, anti-inflammatory and anti-bacterial activity.

Date palm fruits are one of the most popular fruits packed with an impressive list of essential nutrients, vitamins, and minerals that are required for normal growth, development and overall well-being. They contain health benefiting poly phenolic antioxidants<sup>5</sup>.

Dates, fruits of the date palm (*Phoenix dactylifera L.*) are a main source of staple food in

\* To whom all correspondence should be addressed.  
Tel: + 2 01117887429;  
E-mail: mohassan81@gmail.com

arid and semi-arid regions of North Africa, middle east and South-Asian countries. Dates have always played an important role in the economic and social lives of people of this area. Algeria is ranking the fifth world producer of date palm fruits with production about 710,000 tons occupying an area of 170000 hectares<sup>6</sup>. There are over 58 different varieties of dates in region of Ouargla<sup>7</sup>. Moreover, recent Studies have shown that date fruits are an excellent source of phenolics and therefore possess an extremely high antioxidant capacity. Dates have potent anthocyanins, carotenoids, and phenolics compounds (protocathechuic, p-hydroxy benzoic, vanillic, syringic, caffeic, coumaric, ferulic, hydroxy benzoic, mainly cinnamic acids) and flavonoids (flavones, flavonols and flavanones). As of today, dates also have the unique distinction of being the only food to contain flavonoid sulfates, which like most other fruits, have antioxidant properties<sup>8</sup>.

*Phoenix dactylifera L.* commonly known as date palm is one of the oldest and most popular fruit trees in the hot arid regions of the world, particularly in the Gulf countries of the Middle East<sup>9</sup>. Date fruits are a good source of essential nutrients, including sugars, proteins, fibers, trace elements, etc., and form an important part of the daily diet<sup>10</sup>. They are also popular in other parts of the world due to their delicious taste, nutritional value and health benefits. Although ripe and mature sun dried dates are consumed throughout the year, but during the holy month of Ramadan their consumption increases many folds as most of the Muslims break their fast with dates<sup>11</sup>. It is considered to be an important subsistence crop in dry and semi dry regions of the world due to its socioeconomic and traditional importance<sup>12,13</sup>.

Based on the traditional Arabic practice and accepted international terminology, dates are classified into five stages viz. Hababouk (first stage which lasts for 4-5 weeks post fertilization), Kimri (green stage, lasts for 9-14 weeks), Khalal (color stage), Rutab (soft ripe stage) and Tamar (full ripe stage)<sup>14</sup>. Dates are available in different shape and size. The ripe dates are; oval-cylindrical in shape, usually 3- 7 cm long, 2-3 cm diameter and bright red to bright yellow in color depending on the variety. Dates contain a seed (pit) which is about 2-2.5 cm long and 6-8 mm thick and is approximately 13-15% of date's weight<sup>15</sup>. However, fruit quality is

influenced by many factors such as size, color, texture, cleanliness, freedom from defects and the effects of decay-causing pathogens<sup>16,17</sup>.

Dry dates contain approximately 70% of carbohydrates in the form of simple sugars that makes them high energy food. In addition to the macromolecules and other essential micronutrients, phytochemicals like flavonoids, carotenoids, phenolic acids, sterols, procyanidins, and anthocyanins are also present in the dates<sup>18</sup>. Because of these antioxidant phytoconstituents, dates are used in traditional system of medicine for the treatment of hypertension, atherosclerosis, microbial infections, constipation, diabetes and cancer<sup>19</sup>. Consumption of dates on a regular basis is even considered to be beneficial in increasing sexual stamina, reducing sterility caused by various sexual disorders, decreasing fatigue and sluggishness in anemic patients<sup>20</sup>. (Quantification of phenolic compounds, evaluation of physicochemical properties and antioxidant activity of four date (*Phoenix dactylifera L.*) varieties of Oman).

Encapsulation is a process in which thin films, generally of polymeric materials are applied to little solid particles, liquid or gases droplets. This method is used to trap active components and release them under controlled conditions. Several materials have been encapsulated in the food industry, among others, aminoacids, vitamins, minerals, antioxidants, colorants, enzymes and sweeteners<sup>21</sup>.

The main objective of encapsulation is to protect the core material from adverse environmental conditions, such as undesirable effects of light, moisture, and oxygen, thereby contributing to an increase in the shelf life of the product, and promoting a controlled liberation of the encapsulate<sup>22</sup>.

The encapsulating agents used in this study were carrageenan, carboxymethyl cellulose (CMC) and alginate. They have several polar groups such as -OH and -NH which can act as electron donors<sup>23</sup>.

## MATERIALS

Sodium alginate was purchased from Sigma-Aldrich, (Germany). Calcium chloride (CaCl<sub>2</sub>) and potassium chloride KCl were purchased from

Park Scientific limited (UK). *K*-Carrageenan was purchased from Sigma-Aldrich, (Germany).

Carboxy Methyl Cellulose (CMC) was purchased from Sigma-Aldrich, (Germany).

Ferric chloride ( $\text{FeCl}_3$ ) was purchased from Sigma-Aldrich, (Germany).

Gallic acid, Caffeic acid, *p*-Coumaric acid, Vanillic acid, Syringic acid (Figure 1), DPPH, Sodium carbonate, Foline Ciocalteu's reagent and methanol were purchased from SigmaAldrich, USA. All other chemicals and reagent used were obtained locally and were of high purity.

#### Methods

The date fruits were pitted and sliced to pieces with the approximate dimensions of  $1\text{cm} \times 1\text{cm} \times 0.3\text{cm}$ . Then the prepared date fruits were used extraction methods.

#### Sample collection

Five different varieties of *P. dactylifera* L. namely collected from different places from Saudi Arabia. Palm dates fruits of uniform size, free of damage, insects and fungal infections were selected and used for all experiments. Samples were washed with tap water and the pits were removed by hand for preparation of water extract.

#### Extraction of date syrup with water

Date pulp (30 gm) putted in an Erlenmeyer flask (250 ml) and 100 ml of water were added and samples were blending using a hand-held blender (Phillips, Holland). The pH was adjusted to  $6.0 \pm 0.2$ . Each sample, in triplicate, was placed in water bath at  $70^\circ\text{C}$  for 2 h. After heating the slurry was filtered through a cheese cloth with a hand press to remove large impurities and insoluble matters, then the supernatant was decanted and weighed<sup>24</sup>.

#### Preparation of polymer – date syrup beads

##### Alginate – date syrup beads

Sodium alginate was dissolved in water extract of date to produce polymer solutions with a concentration of 2 % (w/v), the solutions was left standing for 3 hrs. to disengage bubble before use. Fifty milliliters of alginate-date emulsion was then sprayed into a collecting water bath containing calcium chloride solution 2% (w/v) by using an Inotech Encapsulator (figure 2) (Switzerland) with a  $450\ \mu\text{m}$  nozzle. The resulting microcapsules were allowed to harden in the  $\text{CaCl}_2$  solution for 3 hrs. Alginate-date beads were collected from the cross-linking solutions using a sieve. Finally, the microbeads were rinsed twice with distilled water;

tissue paper was used to absorb the surface excessive water onto the wet microcapsules<sup>25</sup>.

##### K- Carrageenan - date syrup beads

*K*-Carrageenan was dissolved in water extract of date at  $65^\circ\text{C}$  to produce polymer solution with a concentration of 2 % (w/v), the solution was left standing for 3 hrs. to disengage bubble before use. Fifty milliliters of *K*-Carrageenan-date emulsion was then sprayed into a collecting water bath containing 2% (w/v) potassium chloride solution by using an Inotech Encapsulator (figure 2) (Switzerland) with a  $450\ \mu\text{m}$  nozzle. The resulting microcapsules were allowed to harden in KCl solution for 3 hrs. The beads were collected from the cross-linking solutions using a sieve. Finally, the microbeads were rinsed twice with distilled water; tissue paper was used to absorb the surface excessive water onto the wet microcapsules<sup>26</sup>.

##### CMC - date syrup beads

Carboxy Methyl Cellulose (CMC) was dissolved in water extract to produce polymer solution with a concentration of 2 % (w/v), the solution was left standing for 3 hrs. to disengage bubble before use. Fifty milliliters of CMC-date emulsion was then sprayed into a collecting water bath containing 0.05 M ferric chloride solution by using an Inotech Encapsulator (figure 2) (Switzerland) with a  $450\ \mu\text{m}$  nozzle. The resulting microcapsules were allowed to harden in  $\text{FeCl}_3$  solution for 3 hrs. The CMC beads were collected from the cross-linking solutions using a sieve. Finally, the microbeads were rinsed twice with distilled water; tissue paper was used to absorb the surface excessive water onto the wet microcapsules<sup>27</sup>.

#### Determination of total phenolic content

The total phenolic content was determined according to the Folin-Ciocalteu procedure<sup>28</sup>. Briefly, the extract was transferred into a test tube and the volume adjusted to 3.5 ml with distilled water and oxidized with the addition of  $250\ \mu\text{l}$  of Folin-Ciocalteu reagent. After 5 min, the mixture was neutralized with 1.25 ml of 20% aqueous  $\text{Na}_2\text{CO}_3$  solution. After 40 min, the absorbance was measured at 725 nm against the solvent blank. The total phenolic content was determined by means of a calibration curve prepared with gallic acid, and expressed as milligrams of gallic acid equivalent (mg GAE) per gm of sample. Additional dilution was done if the

absorbance value measured was over the linear range of the standard curve.

#### Determination of radical DPPH scavenging activity

Free radical scavenging capacity of extracts were determined using the stable 1, 1-Diphenyl-2-picryl-hydrazyl (DPPH<sup>•</sup>) according to Hwang and Do Thi (2014)<sup>29</sup>. The final concentration was 200  $\mu$ M for DPPH<sup>•</sup> and the final reaction volume was 3.0 mL. The absorbance at 517 nm was measured against a blank of pure methanol at 60 min.

## RESULTS

#### Total phenolic content in five date cultivars

The total content of phenolic compounds in date cultivars is expressed as mg of gallic acid equivalent in 30 g fresh weight and is presented in table 1.

Total phenol as Caffeic acid equivalent (CAE) is expressed as Mean SD, n  $\frac{1}{4}$  3; Means within a column with no common letter differ

**Table 1.** Total phenolic content in five date cultivars

Type of variety	Total phenolics(mg of gallic acid equivalent / 30 gm fresh wt.)
A	57.1 $\pm$ 0.02
B	57.8 $\pm$ 0.03
C	56.9 $\pm$ 0.15
D	56.3 $\pm$ 0.01
E	55.2 $\pm$ 0.02

A: Barhi, B: Barni Al Madina, C: Khesab, D: Sukkary and E: Ajwa.

significantly ( $p < 0.05$ ). Based on the previous results, the Barni Al Madina was the highest variety of the total phenolics.

#### The antioxidant activity in five date cultivars

The antioxidants activities of five date cultivars were investigated by commonly used DPPH radical scavenging method. The scavenging effect of hydroalcoholic date extracts on the DPPH is expressed as % inhibition. The antioxidant activity equivalent in 30 g fresh weight and is presented in table 2.

According to the results recorded in the above table, it was found that, the Barni Al Madina was the highest activity among the tested varieties.

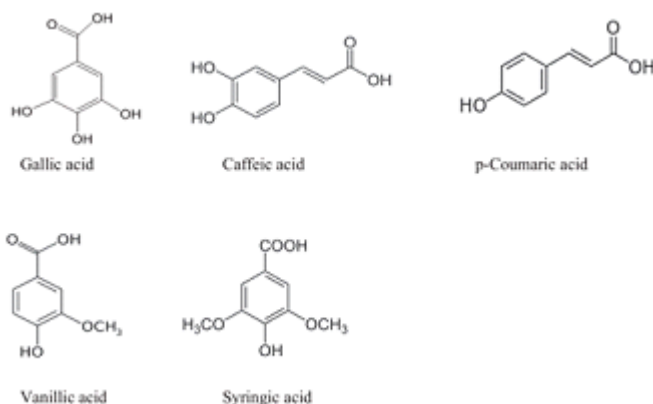
#### Total phenolic content for immobilized date extract

Total phenolic content of immobilized date extract were investigated according to the Folin-Ciocalteu procedure. And from the results shown in figure (3) we notice that the best carrier for encapsulation of date Palme extract is CMC this is may be due to it has more carboxylic groups, then alginate which possess beside carboxylic groups it has also more hydroxyl groups than carrageenan.

**Table 2.** The antioxidant activity in five date cultivars

Type of variety	The antioxidant activity / 30 gm fresh wt)
A	31.1 $\pm$ 0.01
B	31.8 $\pm$ 0.02
C	30.9 $\pm$ 0.04
D	29.3 $\pm$ 0.12
E	29.2 $\pm$ 0.02

A: Barhi, B: Barni Al Madina, C: Khesab, D: Sukkary and E: Ajwa.



**Fig. 1.** Phenolic acids

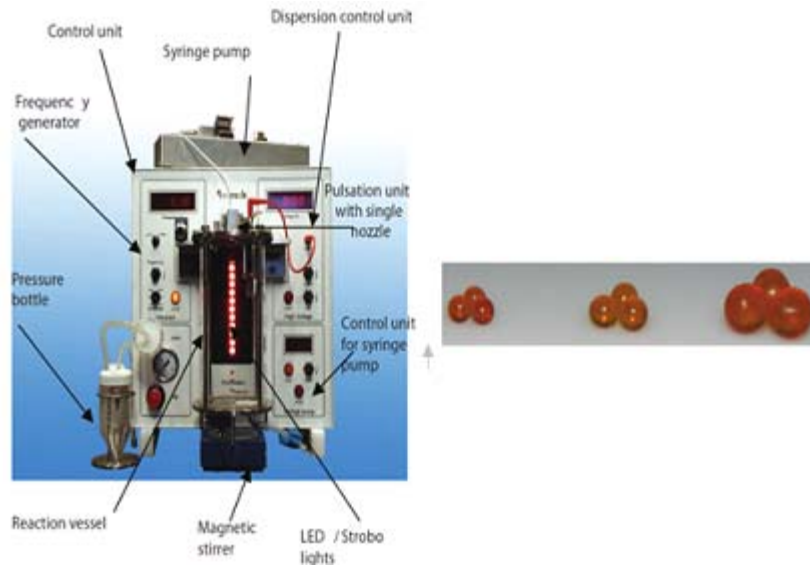


Fig. 2. Encapsulator for making uniform gel beads

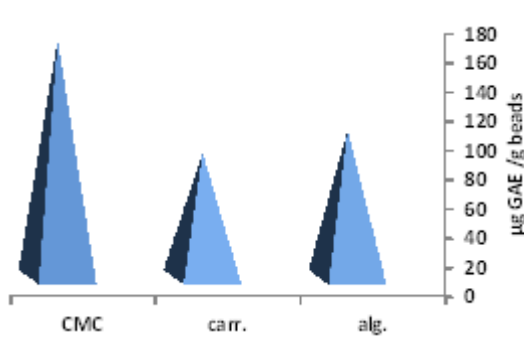


Fig. 3. Total phenolic content for immobilized date extract

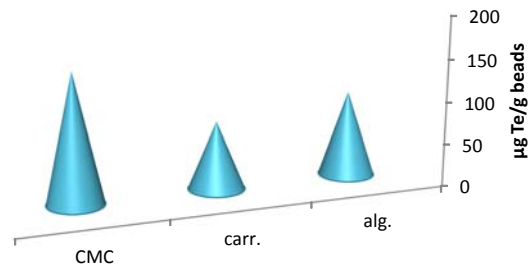


Fig. 4. The antioxidant activity for immobilized date extract

### The antioxidant activity for immobilized date extract

The antioxidant activities of immobilized date extract were investigated by commonly used DPPH radical scavenging method. The scavenging effect of hydroalcoholic date extracts on the DPPH is expressed as % inhibition. And from the results shown in figure (4) we notice that the best carrier for encapsulation of date Palme extract is CMC this is may be as it has more carboxylic groups, then alginate which possess beside carboxylic groups it has also more hydroxyl groups than carrageenan

### DISCUSSION

The total phenols assessed using Folin–ciocalteu phenol’s reagent showed that the Barni Al Madina possessed significantly higher amount of phenolics than the other types of cultivars with the values of 57.8 mg of gallic acid equivalent (Table 1). At the cultivar level, total phenolics ranged from 57.8 and 55.2 mg of gallic acid equivalent /30 g fresh wt. Estimates of total phenolics in dates using the colorimetric Folin–Ciocalteu method varies greatly according to variety<sup>11</sup> and phenolic standards used as well as the units used to express the data. Our data are in agreement with previous

studies indicating that phenolic content in fruit of date palm decrease as ripening progressed<sup>11, 31</sup>. Thus, Awad *et al.* founded total phenolics concentrations in the range of 50 and 10 mg/100 g fresh weight (FW), in fruits of five date palm cultivars grown in Saudi Arabia<sup>32</sup>. In addition, the degree of ripeness considerably affects the concentrations and proportions of the various total phenols in edible plants. In general, it has been observed that phenolic acid concentrations decrease during ripening<sup>33</sup>.

The antioxidant activity of fruit extracts was assessed using the DPPH assay. The study revealed that cultivars of the Barni Al Madina exhibited the highest antioxidant activity with an average of 31.8 (%) /30 g FW. Also we found that the antioxidant activity was ranging from a low value of 29.2 in Sukkary cultivar to a high value of 31.8 in Barni Al Madina cultivar (Table 2). The antioxidant activity of dates was reported by several authors<sup>34,35</sup>. Although different assays has been used in the assessment of their antioxidant activity such as DPPH, ferric-reducing antioxidant potential (FRAP), 2,20-azino-bis (3-ethylbenzthiazoline)-6-sulfonic acid (ABTS), they all concluded to the powerful antioxidant activity of date fruit at both ripening stages. Mansouri *et al.* using the same assay to estimate the antioxidant activity expressed as the mass ratio (1g sample/1g DPPH) in seven different ripe date palm fruits from Algeria, including Deglet nour, founded an antiradical efficiency that ranged from 0.08 to 0.22.<sup>36</sup>

### CONCLUSION

Date palm (*Phoenix dactylifera L*) is an important fruit and cash crop in Saudi Arabia, it is socioeconomically and traditionally important for local populations where the culture thrives. Saudi Arabia, being a vast country, has a number of varieties of dates differing. But, unfortunately, it is still to be explored. The results presented in this study showed that the date palm fruit from Saudi Arabia has antioxidant activity and phenolic content. On the basis of our finding, we conclude that date palm fruit constitutes a natural source of potent antioxidants that may prevent many diseases and could potentially be used in food and nutraceutical formulations. In the other hand it can be encapsulated in different types of bio

polymeric matrix and from the data obtained we found that CMC is the best one then alginate and carrageenan come last one.

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