



Phenological Stages as Quality Determinants of Phenolics Content and Antioxidant Activity of Seven Date Varieties Grown in Algeria

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ABSTRACT

In the present study, the methanolic extracts of fruits of seven date palm varieties (Deglet-Nour, Mech-Deglet, Ghers, Arechti, Ytima, Taouri, Tantboutche) grown in Biskra, Algeria were assessed for their total phenolics, and flavonoid content and antioxidant activity at three edible maturity stages namely Khalal, Rutab and Tamar according to the standard Iraqi Arabic nomenclature. Polyphenols expressed as mg of gallic acid equivalents (GAE) per g of dry matter (DM) were found to be relatively higher in Khalal (1.705 - 8.620), from 0.303 to 4.787 in Rutab, and from 0.161 to 1.993 in Tamar. On the other hand, the flavonoids content calculated as quercetin equivalents (QE) per g of DM was found to vary between 0.282 and 0.627 in Khalal, from 0.210 to 0.491 in Rutab and from 0.008 to 0.075 in Tamar. Moreover, the antioxidant activity was conducted using DPPH (2,2-diphenyl-1-picrylhydrazyl) free radical assay. Important scavenging ability was recorded with almost all varieties in the Khalal and Rutab stages (average IC_{50} of 30.417 μ g/ml for Khalal and 30.415 μ g/ml for Rutab), however the Tamar fruits displayed the weakest activity (average IC_{50} =107.00 μ g/ml). A positive correlation was observed between total phenolics and antioxidant ability in different stages and varieties suggesting that phenolics were the main contributor to this activity.

Keywords: *Phoenix dactylifera*; date fruit; phenological stages; Total Phenolic; flavonoids; Antioxidant activity

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INTRODUCTION

The date palm (*Phoenix Dactylifera*) is one of mankind's oldest cultivated plants that has been used as food for about 6,000 years (Sahari et al. 2007). It is cultivated mainly in North Africa but also in South Asia, in the USA and in Australia. *Phoenix Dactylifera* covers a surface of about 800,000 ha and it is important-directly or indirectly-for the life of about 100 million inhabitants (Ghiaba et al. 2012).

The date (*Phoenix dactylifera* L.) is the fruit of the date palm, produced in the Saharan regions and considered a food of great importance for the population living in these regions. Algeria with its rich and diverse date palm heritage, over 13 million palm trees and 940 cultivars is recorded with a total date production estimated at 440 000 tonnes is one of the world's largest producers of date, ranking 7th worldwide (Hannachi et al. 1998).

The chemical composition and nutritional value of date was well studied (Al-Farsi et al. 2005; Al-Shahib et al. 2002, Fayadh

et al.1990). Date is some high energy food rich in carbohydrates, a good source of minerals, such as calcium, iron, magnesium, phosphorus, potassium and zinc; but, it is low in fats and proteins (Hong et al. 2006, Periyasamy et al, 2017). Besides nutritional compound, date is rich in antioxidants. Antioxidant activity of this fruit from different origins (Oman, Iran, Algeria, Kuwait, etc) has been studied by several authors (Biglari et al. 2008; Mansouri et al. 2005). These biologically active compounds, which can quench reactive free radicals, such as superoxide radical, hydroxyl radical, hydrogen peroxide, can prevent the oxidation of other molecules such as proteins and lipids (Atmani et al. 2009). Therefore, through reducing the risk of major chronic health problems, an important role is played by antioxidants in human health.

2. MATERIAL AND METHODS

2.1. Sample Preparation

Seven varieties of Algerian dates, namely: Deglet-Nour (fig 1), Mech-Deglet, Ghers, Arechti, Ytima, Taouri and Tantboutche were collected during three maturity stages (Khalal, Rutab and Tamar according to the standard Iraqi Arabic nomenclature) from the Bentious location, 45 km from Biskra city southeast of

Algeria. Ten (10) healthy fruits of each date variety were subjected to drying process. The samples were weighed before

and after the drying process to estimate the water content.



Deglet-Nour(Khalal stage)



Deglet-Nour (Rutab stage)



Deglet-Nour (Tamar stage)

Figure 1. a sample of Deglet-Nour variety at different phenological stages

2.2. Preparation of the Extracts

The date material was air dried in shade at room temperature, and crushed into a fine powder using a coffee-grinder. The phenolics content was assessed using a modified version of the method described by Djeridane (Djeridane et al, 2006). 20 g of dry dates were macerated in 100 ml methanol for 24 h at room temperature. The crude preparation was filtered, and the residue was re-extracted twice with 50 ml of the same alcoholic solvent for 24 h at room temperature. The extract was filtered and the alcoholic filtrates were combined and evaporated under vacuum at 40 °C.

2.3. Total phenolics content (TPC)

The polyphenols content of the methanolic extract was determined using the Folin-Ciocalteu method (Singleton and Rossi, 1965). 300 µL of the sample were added to 1500 µL of Folin-Ciocalteu and 1200 µL aqueous sodium carbonate Na₂CO₃ (7.5%). The mixture was incubated for 1 hour and the absorbance of total phenols was read at 760 nm using a spectrophotometer (SHIMADZU UV1650PC). The standard curve was prepared using 0.02, 0.05, 0.1, 0.15, 0.5 µg/mL of gallic acid solutions in methanol. Total phenols (TPC) were determined from standard gallic acid curve, expressed as milligrams of gallic acid equivalents per g of dry weight plant material (GAE/g DW).

2.4. Total flavonoids (TFC)

The total flavonoids content was determined according to the aluminum chloride colorimetric method described by Chang (Chang et al, 2002) with slight modifications. The results are expressed as mg of quercetin equivalents per 100 g of dry weight (QE/100 g DW). This method is based on the quantification of yellow colour produced by the interaction of flavonoids with AlCl₃ reagent. To 1500 µL of each sample is added 1500 µL of AlCl₃ (2% (w/v) in methanol solution and incubated for 10 min in the shade at the room temperature. The absorbance was measured at 430 nm using SHIMADZU UV1650PC-UV-visible spectrophotometer. TFC was determined from standard quercetin curve and expressed as mg of quercetin equivalents per g of dry weight of plant material (QE / g DW).

2.5. Antioxidant Activity

Determination of the free radical scavenging activity of the different extracts was carried out using a modified quantitative DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. Various concentrations of extracts in methanol were prepared (50, 100, 150, and 200 µg·ml⁻¹). Blank samples were run using 1 mL methanol in place of the sample. 1 mL of 0.004% DPPH in

methanol was added to 1 mL of the test solution, or standard, and 1 ml of methanol for dilution. The mixture was allowed to stand at room temperature in a dark for 30 min. The change in colour from deep violet to light yellow was then measured at 517 nm. Inhibition of free radical in percent (I%) was calculated according to the following equation: $I\% = \frac{(A_0 - A_1)}{A_0} \times 100$, with A₀ being the absorbance of the control reaction (containing all reagents except the sample) and A₁ the absorbance of the extract. Extract concentration providing 50% inhibition (IC₅₀) was calculated from the graph plotted of inhibition percentage against extract concentration. Measurements were carried out in triplicates.

3. RESULTS AND DISCUSSION

3.1. Moisture content:

Important fluctuations of water content were observed depending on the ripening stage and the date variety. Indeed, Khalal stage is characterised by a relatively higher water fraction, ranging from 62 to 77% (w/w). In Rutab stage, however, the water content decreased and varied between 37 and 49%. Tamar stage, in turn, has the lowest water content varying from 14 to 26% (Table 1). This is the last maturity stage and dates are ripe enough to be collected and used as food.

Table 1. moisture rate in seven date palm varieties fruits at three maturity stages

Date variety	Khalal	Rutab	Tamar
Deglet-Nour	70.83	49.90	26.67
Mech-Deglet	62.23	37.13	14.17
Ghers	74.21	43.13	22.00
Arechti	77.84	42.77	19.67
Ytima	72.06	51.69	23.17
Taouri	65.80	43.57	17.08
Tantboutche	68.85	44.62	24.56
average	70.26	44.69	21.05

3.2. Methanolic Yield

The methanolic yield varies from one sample to another and from one stage to another as presented in the Table 2. In Khalal stage, the average methanolic yield was comparatively lower with a minimum yield in Taouri date variety (15.50%). On the

hand, a maximum yield was recorded at Tamar stage in Mech-Deglet (73.50%).

Table 2. % Yield of methanolic extraction (w/w) of the studied varieties at the three stages based on dry weight

Date variety	Khalal	Rutab	Tamar
Deglet-Nour	19.00	20.66	44.20
Mech-Deglet	16.00	17.00	73.50
Ghers	23.00	38.66	46.28
Arechti	25.50	57.00	50.40
Ytima	22.50	55.00	54.70
Taouri	15.50	64.33	45.60
Tantboutche	18.50	36.00	50.80
average	20.00	41.24	52.21

3.3. Polyphenols content:

Polyphenols are plant secondary metabolites of capital importance by virtue of their antioxidant activity in chelating redox active metal ions, inactivating lipid free radical chains and preventing hydroperoxide conversions into reactive oxy-radicals.

• **Total phenolics content (TPC)**

The total phenol content (TPC) in methanolic extracts fruits was assessed using the Folin-Ciocalteu assay as mentioned previously (Table 3). Polyphenols expressed as mg of gallic acid equivalents (GAE) per g of dry matter (DM) were found to be relatively higher in Khalal (1.705 - 8.620), from 0.303 to 4.787 in Rutab, and from 0.161 to 1.993 in Tamar. The TPC values in Khalal stage varied as follows: Ghers > Taouri > Tantboutche > Ytima> Mech-Deglet > Deglet-Nour > Arechti. In Rutab stage, the TPC varied as follows: Tantboutche> Deglet-Nour > Mech-Deglet > Arechti > Ytima > Ghers > Taouri. Tamar stage in turn involves relatively lower TPC values are ranked as follows: Mech-Deglet > Ghers > Tantboutche > Taouri > Arechti > Deglet-Nour> Ytima. The TPC contents found in the study in hand are unlike those reported in many preceding investigation (Ghiaba et al. 2012). Diverse causes might interfere in the observed discrepancies such soil texture, climatic conditions, geographic location, storage conditions, fertilizer, drying process, cultural techniques, extraction solvents and methods and so forth.

• **Total flavonoids content (TFC)**

The total flavonoids content of various date extracts is presented in Table 3. Similar to TPC, the TFC was averred more important at Khalal stage than at the other stages. In fact, during Khalal Stage, the TFC with an average value of 0.460 mg of Quercetin equivalent/g of DW, followed the order: Mech-Deglet (0.627) > Ghers>Arechti>Tantboutche>Deglet-Nour>Ytima>Taouri (0.282). Nevertheless, at the Rutab stage the average TFC was found to be 0.348 mg of Quercetin equivalent/100g of DW and the arrangement is a bit different: Tantboutche (0.491) > Deglet-Nour>Taouri>Ytima>Arechti>Mech-Deglet>Ghers (0.210). At Tamar stage the lowest TFC values were recorded following the subsequent order: Taouri (0.075)>Deglet-Nour>Ghers>Arechti>Ytima>Tantboutche>Mech-Deglet (0.008).

Table3. Total phenolics content in mg GAE/g of DW and total flavonoids content in mg QE/g of DW

Date variety	Khalal		Rutab		Tamar	
	TPC	TFC	TPC	TFC	TPC	TFC
Deglet-Nour	2.050	0.389	1.860	0.431	0.330	0.070
Mech-Deglet	2.480	0.627	1.493	0.243	1.993	0.008
Ghers	8.620	0.600	0.307	0.210	0.850	0.055
Arechti	1.705	0.531	1.127	0.295	0.360	0.054
Ytima	3.500	0.334	1.123	0.375	0.161	0.051
Taouri	5.620	0.282	0.303	0.395	0.581	0.075
Tantboutche	4.820	0.457	4.787	0.491	0.684	0.048
average	4.113	0.460	1.571	0.348	0.708	0.051

3.4. DPPH radical-scavenging activity

1.1-diphenyl 2-picrylhydrazyl (DPPH) is a free radical, that involves an odd electron and is used to estimate the ability of date palm varieties to act as free radical scavenger through the change in its absorbance produced by the reduced form. Each methanolic extract was subjected to DPPH assay. IC₅₀ values were recorded for all extracts as shown in table 4 and fig 2. Comparing the activities of different extracts, the highest effect designated by the lowest IC₅₀ (10.342 µg/mL) value was recorded in the Taouri variety at Rutab stage extract which varied in the following order: Taouri> Deglet-Nour > Ytima > Ghers > Arechti > Tantboutche > Mech-Deglet.

Table 4. IC₅₀ values in µg/mL for each methanolic extract at different maturity stages against DPPH

Date variety	Khalal	Rutab	Tamar
Deglet-Nour	24.624	13.078	68.873
Mech-Deglet	23.677	99.287	17.017
Ghers	32.377	16.648	112.705
Arechti	46.479	24.038	141.367
Ytima	29.493	13.494	289.506
Taouri	27.406	10.342	38.985
Tantboutche	28.865	36.017	80.604
Average	30.417	30.414	107.008

On the other hand, at Khalal stage. the date palm varieties displayed the following order of scavenging ability: Mech-Deglet>Deglet-Nour>Taouri>Tantboutche>Ytima>Ghers>Arechti. Al last at Tamar stage. The antiradical potency was ranked as follows: Mech-Deglet> Taouri > Deglet-Nour>Tantboutche> Ghers > Arechti > Ytima. In previous reports, it was stated that all the phenolic classes are gifted with remarkable biological properties including scavenging activity (Ben Slima et al. 2013).

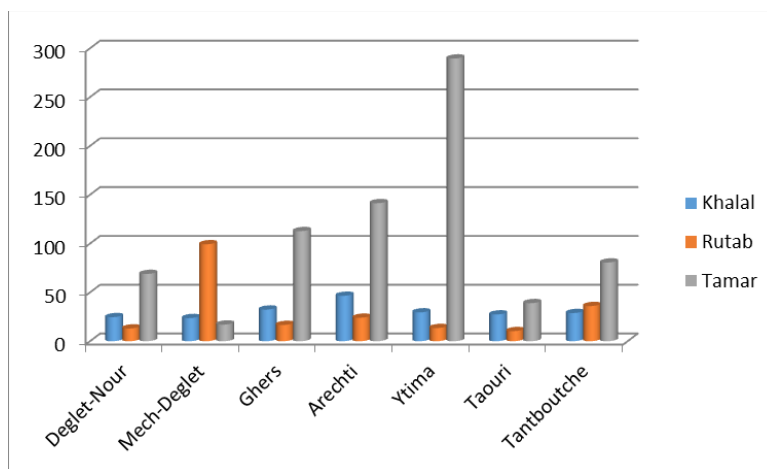


Figure 2. antiradical activity (IC_{50}) values for each plant extract at different maturity stages against DPPH (as shown Taouri variety is the most potent at Rutab stage) and Ytima is the less potent at Tamar stage.

CONCLUSION

Methanol extracts of seven Algerian date palm varieties were investigated for their phenolic content and radical scavenging activity at three ripening stages. The results established that the highest phenolic content was in Ghers variety at Khalal stage whereas, the lowest value was recorded in Mech-Deglet variety at Tamar stage. Moreover, all the tested date fruits exhibited antiradical activity against DPPH ranging from relatively high potency (indicated by the lowest IC_{50} value) in Taouri variety at Rutab stage to quite weak activity in Ytima variety at Tamar stage. An apparent correlation could be observed between the phenolic content and antiradical ability. Therefore, this widely consumed food can be regarded as an effective natural antioxidant source especially at Khalal and Rutab stages. Other in-vitro and in-vivo studies are necessary especially on date palm by-products to valorise this plant as alternative to industrial additives in cosmetic and food manufacturing.

Conflict of Interest: The authors declare no conflict of interest.

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