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A comparative study of total phenolics, antioxidant activity and anthocyanins of three Algerian dates varieties

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Abstract

The objective of this study was to compare phenolic profile of three varieties of Algerian dates, and the use for the first time of the oxygen radical absorbance capacity (ORAC-FL) to determine antioxiadant capacity of dates. Total phenolics using Folin-Ciocalteu reagent and the anthocyanins by the pH differential method in three varieties of dates (*Dglet-Nour, Ajwa* and *Ghars*). The results obtained were expressed as means of three replicate ± standard deviation (n = 3) on a fresh weight basis. Total polyphenolics values were about 97.16 ±1.25 and 245.32 ± 13.12 and 246 mg/100 g Ferrulic Acid Equivalent (FAE) for *Deglet-Nour* variety (water/aceton extraction) respectively. In the same order of solvents used. The varieties (*Ghars* and *Ajwa*) have presented total phenolics concentrations: (73.81±0.81; 86.63 and 64.77±0.42; 81.11) mg/100 g FAE. These dates' varieties have shown a good result of total anthocyanins (0.91; 0.75 and 0.56 mg equivalent cyanidine3-glucoside /100g for the varieties *Deglet-Nour*, *Ajwa* and *Ghars* in the same order. ORAC-FL have presented a high results compared by other methods ((DPPH, ABTS, FRAP and TEAC). High values were registred for *Deglet-Nour* (4844 and 4818.67 ±32.14) µ moles equivalent trolox/100 g and 3891µ moles for *Ajwa*. Only 2648 µ moles equivalent trolox /100 g were registered for *Ghars* variety. The temperature effect on pH and total phenolics content was equally tested in this study. In this work, we have added a new conclusion about the high nutrition value of Deglet-Nour variety.

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Introduction

The date fruit (*Phoenix dactylifera* L.) is a widely consumed fruit in warm and semi-arid regions (Middle East and North Africa). The composition of palm date varies with the harvest period and environmental conditions (Tang *et al.*, 2013). In advanced stages of maturation, the date is very rich in antioxidants, especially polyphenols. That is why we have undertaken to carry out this work on three varieties of Algerian dates (Deglet-Nour, Ghars and Ajwa) to assess the levels differences of total polyphenols, anthocyanins and the antioxidant activity. In this work, we evaluated the antioxidant activity by the method of the ORAC-FL, which has not been used before for these varieties of dates. Other compounds (anthocyanins) are also important in the study of key molecules in relation to the antioxidant power and are poorly studied and discussed among literature. On the other hand, two extraction solvents; hot water and acetone: water and the storage temperature have been studied on the total phenolic content of the three varieties of dates were also the subject of study in order to show if these parameters can affect on the date phenolic profile.

Algeria is ranked fourth in the world in terms of date production with annual production of about 789 tons of dates during the year 2015 and that Deglet-Nour variety represents more than 50% and these are statistics according to the FAO 2015. This variety is highly valued by consumers, and is exported mainly to European countries. According to the Algerian national office of dates, dates production has increased from 5528 to7249 quintals between 2008 and 2014 (Belghoul Y, Benmehidi O, 2015). Deglet-Nour is a commercial variety of choice while common varieties are of lesser economic importance and the most common of which are Degla-Beida and Mech-Degla. Research on the effects on health polyphenols started much later than other antioxidants. This is largely explained by the great diversity of chemical structures. Polyphenols are able to trap free radicals that are generated continuously by the body or formed in response to the attacks of our environment (smoke, pollutants, infections, etc.) which are directly related to several diseases (Al-Turki *et al.*, 2010).Various studies have been conducted to determine the chemical composition of dates, sugars, proteins, fats, fiber, vitamins and minerals. Studies on the polyphenol composition are on a few varieties of dates. In addition, the results published on the elementary chemical and biochemical analysis of dates are varied and sometimes have highly significant differences (if dosing of phenolic compounds and antioxidant activity) this can be explained, by the differences of the methods used, the extraction solvent and the operating conditions.

Materials and methods

Plant origin and preparation

Three varieties of dates (*Phoenix dactylifera* L.) used in this work were of an Algerian origin: Deglet-Nour from Biskra region, Ghars and Ajwa varieties from Ouargla (Fig. 1). Dates are first pitted and then finely ground to obtain a well mixed paste to be used for the extraction of phenolic compounds and further analysis.

Total phenolics extraction methods

The varieties of dates were extracted with distilled water and aceton/water (70:30). The extraction method with hot water is to soak the sample (10 g of crushed pulp dates/100 ml of hot distilled water at 60°Cfor 3 hours and 30 minutes with three successive extractions, keeping out of light to obtain maximum extraction of total polyphenols (Al-Farsi *et al.*, 2005; Kchaou *et al.*, 20013). For aceton/water (70/30) extraction, we proceeds to macerating 10 g of dates in 100ml of solvent for two hours with three successive extractions with exclusion of light, followed by centrifugation 6000 rpm/5 min, filtration and evaporation. The extracts were combined and stored at low temperature (4°C) and kept out of light until analysis.

Total phenolic measurement

Folin-Ciocalteu method for total phenolics has been widely described in the literature (Singleton and Rossi, 1965; Medina-Remon *et al.*, 2009), the same method was used with slight modification.

Products and reagents

Folin-Ciocalteu (Sigma-Aldrich, Germany); sodium carbonate (Merck); ferulic acid and gallic acid (Fluka-Chemica).

To assay the total phenolic content of dates,790 µl of distilled water were added to 10 µl of dates extracted juice, to this solution the addition of 50 µl of the Folin-Ciocalteu reagent (diluted ten times) after five minutes was added 150 µl of saturated sodium carbonate.

After two hours away from light, absorbances were read at the spectrophotometer at a wavelength of 760 nm.

The extraction tests, analysis and reading were repeated three times.

Calibration curves

Ferulic acid standard curve was performed using different concentrations (0.1-0.08-0.07-0.05 and 0.03 mg/ml) from a stock solution of ferulic acid of 0.1 mg/ml.

The regression obtained is y = 0247-0001 (R^2 = 0.987) and the results were expressed in milligrams (mg) of ferulic acid equivalents/100 g sample. Gallic acid standard curve was prepared to obtain a final concentration of 5 g/liter. Derived solutions were prepared in order to obtain concentrations ranging from 50 to 500 mg /liter and the regression equation is given: y = ax + b

Where: Y = optical density of the sample; X= the value of the total polyphenol concentration expressed as mg GAE/100 g sample or in mg ferulic acid equivalent/100 g fresh weight or mg ferulic acid/100 ml of fresh juice(Medina-Remon *et al.*, 2009).

Total anthocyanins

Total anthocyanins were determined according to the pH differential method of (Al-Farsi *et al.*, 2005; Allaith *et al.*, 2012) with a quiet modification (including pH and solutions volume adjusted).

The absorbance was read at 510 nm and at 700 nm and the result is expressed by the equation:

Ab = (A510 nm - A700 nm) pH1 - (A 510 nm - 700 nm) pH 4,2.

Total anthocyanins (mg/100g)= Ab/eL × MW × D × V/G × 100 Equation 3

Ab: Absorbance at 510 nm and 710 nm, e: Molar extraction coefficient (26900).

MW: Molecular weight of anthocyanins (449.2), D: dilution factor, V: final volume (ml), G: sample weight (mg), L: tank wide (1cm).

Antioxidant activity measurement (ORAC assay)

In this study, we have used the method of ORAC-FL (Oxygen Radical Scavenging Activity) described by (Ou *et al.*, 2001; Fernandez Pachon, 2005). To determine the antioxidant activity of a sample. It is necessary to refer to a standard antioxidant, the most used is the Trolox: 6-hydroxy-2,5,7, 8tatramethylcroman- 2-carboxylic acid which is water soluble analogue of α-tocopherol.

The fluorescence was determined (λ excitation: 490 nm and λ emission: 515 nm) every 5 minutes for sixty minutes to an approximate decrease to 0 or a value less than 5% of the initial value.

Products and reagents

Sodium Fluoreseina (Fluka), Trolox, AAPH (Sigma), Ethanol (Merck). The results of the antioxidant activity were calculated using the equation: ORAC micromoles = 20 K (SE - SB)/(ST SB).

Where: 20 is the concentration of Trolox; K is the sample dilution factor; S is the bottom surface of the sample of fluorescein decay curve (E), Trolox (T) and white (B).

The results were expressed in Trolox equivalent (µmol L-1.): Micromol Trolox equivalent/liter of the sample.

Equipment used

Scales Type: AG 204 BELTA Range Mettler-Toledo (0.1 mg -210 mg) Startorius BL. 1500s * max 1500g. Shaker: IKAMAG® REO - Electronic (100-1100 rev/min). Centrifuge: BECKMAN-AVANTIJ. Spectrophotometer: GENESYS 20 thermospectronic Analysis. Microplate spectrophotometer: Beckman - Coulter AD-340 Rotavapor BUCHI K- 200.

Effect of time and storage temperature on pH and on the total polyphenols

Dates extracts were kept at temperatures (-18 °C and 22 °C) within 16 days. For each variety, pH measurements and the determination of total polyphenols were performed at t = 0 days, 7 days and 16 days. Each measurement and analysis was performed in triplicate.

Statistical analysis

Statistical analysis of all results were carried out using Statbox ANOVA; the statistical software of variance analysis. All measurements were performed in triplicate and given as mean Sv± Standard Deviation (SD).The results are significant when $p<0.05$.

Results

Total phenolics results

The results are shown in Table 1 and 2**.** The highest concentrations 246 and 97.16 ± 1.25 (mg AFE/100 g of extract) were recorded for the variety Deglet-Nourmonitoring concentrations of 73.81 ± 0.81 and 86.63 (mg AFE/100 g of extract) and 64.77 ± 0.42 and 81.11 (mg AFE/100 g of extract) for varieties Ghars and Ajwa respectively.

Table 1. Total polyphenols of three varieties of dates: Deglet-Nour; Ghars and Ajwa extracted with aceton/water (70/30). Polyphenols concentrations are given in (mg FAE/100g).

Sample weight (g)	Deglet-Nour	Ghars	Ajwa
50	102,0	36,4	52,1
70	119,0	47,4	63,5
100	246,0	86,6	81,1

Table 2. Total phenolics of three varieties of dates: Deglet-Nour; Ghars and Ajwa, extracted with water.

Sample weight (g)	Deglet- Nour	Ghars	Ajwa
50	33,0	30,7	30,3
70	41,0	38,8	35,6
100	97,2	73,8	64,8

Polyphenols concentrations (mg FAE/100g).

Total anthocyanins

The results of determination of anthocyanin of three varieties of dates on different samples weight (50; 70 and 100g) are shown in Table 3. 0.91mg cyanidins equivalent/100 g fresh weight was recorded for the variety Deglet-Nour, monitoring of both varieties Ajwa and Ghars respectively (0.75 and 0.56 mg cyanidins equivalent/100 g fresh weight).

Aantioxidant activity

The results of the antioxidant activity of three varieties of dates are shown in Table 4 obtained from 3 extractions (50, 70 and 100 grams of dates). We recorded for the variety Deglet-Nour (4352.33 ± 34 µmole Trolox/100 g weight) followed by the varieties Ajwa and Ghars (3891 and 2648)µ mol Trolox/100 g weight.

Storage temperature effect on pH and on total phenolics of dates

The results of the evolution of pH at temperatures (-18 °C and 22 °C) are shown in Fig.2 and Fig.3.At -18 °C (pH = 5.85 to 5.72) for the variety Deglet-Nour, and 5.64 to 5.19 for Ghars and values from 5.89 to 5.09 for the Ajwa variety (Fig.2). pH values at 22°C for the variety Deglet-Nour (6, 19 to 2,61) and (5,78 to 2,82) for Ghars. The variety Ajwa has presented (6 to 2,36) of pH.

These storage parameters (time and temperature)effect on the total phenolic content is reported in (Fig.4,) which present water solvent and stored at -18°C, It was ranged between(97,16-64,91) mg AFE / 100 g of extract for the variet Deglet-Nour and (73,81 to 53,57;64,77 to 53,44) mg AFE / 100 g for Ghars and Ajwa respectively. At 22°C and in final time(16 days), these values were less since the minimum;41,43 for Deglet-Nour and(30,09 and 21,45) mg AFE /100 g for Ghars and Ajwa in same order(Fig.5).While on the Fig.6 the total phenolics extracted with Aceton/water and storage at-18°C have shown quiet difference. Polyphenols results with Aceton/water at the end of storage (16 days) and stored at 22°C are: (56,81;32,92:38,33)mg AFE/100 g for Deglet-Nour, Ghars and Ajwa in same citation.

Table 3. Total anthocyanins (mg equivalent cyanidins 3-glucoside /100g fresh weight).

	Deglet-Nour			Ajwa			Ghars		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
R1	0,78	0,89	0,92	0,65	0,82	0,80	0,36	0,44	0,46
R2	0,77	0,86	0,96	0,69	0,79	0,88	0,41	0,38	0,62
R3	0,75	0,81	0,87	0,81	0,65	0,85	0,36	0,40	0,61
Means ± SD	0,75±0,02	0,85±0,04	0,92±0,05	0,72±0,08	0,75±0,09	0,84±0,04	0,38±0,03	0,41±0,03	0,56±0,09

T1, T2, T3 are samples weight (g) :T1= 50, T2 = 70, T3 = 100

R1, R2, R3 : Repetitions. SD : Standard deviation.

Table 4. Antioxidant activity (µmole Trolox/100g weight).

	Deglet-Nour			Ajwa			Ghars		
	T1	T2	T3	T1	T2	T3	T1	T2	T3
R1	4362	4841	5546	3622	3712	4182	2633	2680	3012
R2	4315	4731	5423	4012	3690	4075	2436	2658	2822
R3	4380	4831	5170	3816	4044	3866	2817	2791	2817
Means ± SD	4352±34	4801±61	5380±192	3817±195	3815±198	4041±161	2353±330	2710±710	2884±111

T1, T2, T3 are samples weight (g) : T1= 50, T2 = 70, T3 = 100

R1, R2, R3 : Repetitions. SD : Standard deviation.

Discussion

In this work, we have produced a good result and we recorded the highest total polyphenol concentrations for the three varieties extracted with acetone/water. For both solvents, the highest concentrations were recorded for the variety Deglet-Nour. This is consistent with the work of Allaith (2008).Our results are similar to those reported by (Al-Farsi *et al.*, 2005; Brad, 2008; Behija Safi *et al.*, 2009;Borchani *et al.*, 2011; Benmeddour *et al.*, 2013; Kchaou *et al.*, 2013) who measured the total polyphenols in dates, grapes, onion and apples.

Phenolic compounds extraction from plant materials is a key step for the various analyzes. For this effect, it was important to choose the most effective method and extraction solvent. Al-Farsi and Lee (2008) have clearly shown a positive effect of these factors (solvent method) on the linear relationship between total phenolic content and volume of solvent. Borchani *et al.* (2011) have analyzed the total phenolic content of a variety of Tunisian dates after lyophilization and achieved a significantly higher value. It was concluded by (Al-Farsi *et al.*, 2005) that fresh dates are a good source of antioxidants comparing with dry varieties and that anthocyanins were detected only in the fresh dates. Most of authors have confirmed that there is a decrease in the total polyphenol content of date according to the stages of maturation. Mrabet *et al.* (2012) have analyzed the total phenolic content and antioxidant activity (aceton: water 70:30) of eleven varieties of Tunisian dates, they reported that the highest concentration was represented by the Tunisian *Degla* variety. Behija Safi *et al.* (2009) have

also reported a good result for fresh variety. Our results showed a total polyphenols relatively high rate comparing with the Tunisian dates and even with other authors (Awad *et al.*, 2011). Concerning anthocyanins, The mean levels of anthocyanins assayed for the three varieties of fresh dates showed a significant difference ($p<0,05$) for the highest content in Deglet-Nour, their chemical structure makes them excellent antioxidant activity.

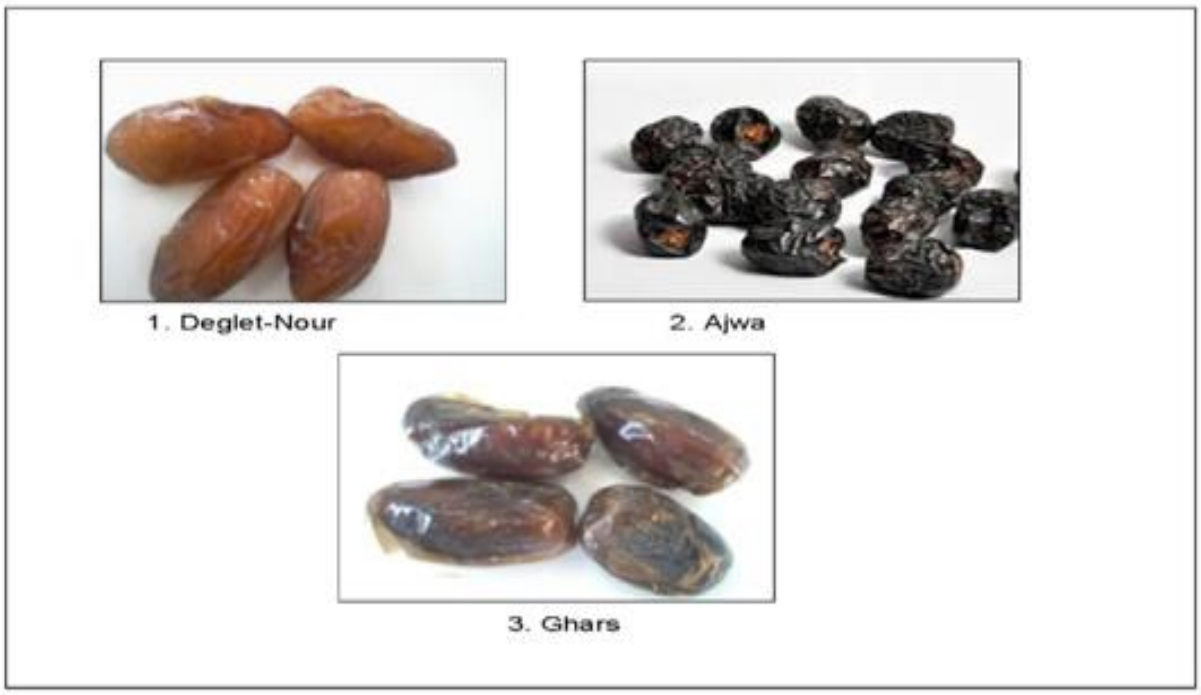

Fig. 1. Varieties of dates used in this study (Deglet-Nour, Ajwa, Ghars).

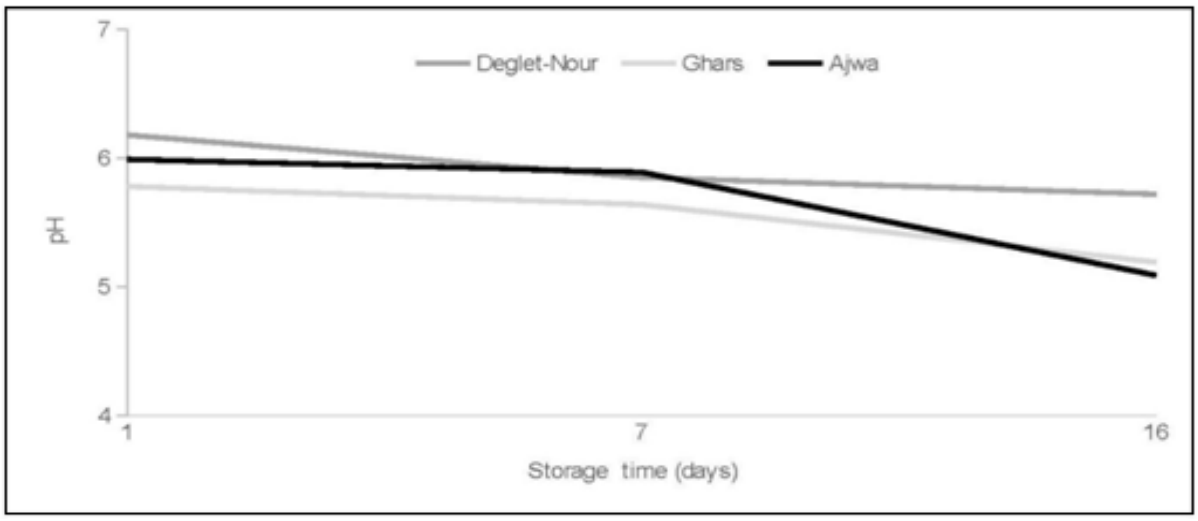

Fig. 2. pH changes of three dates varieties stored at- 18 ˚C during 16 days.

These are flavonoids from secondary metabolism with a molecular weight of 400 to 1200 (He and Giusti, 2010; Martin Bueno *et al.*, 2012).

According to (Collin and Crouzet, 2011), at pH 1, the flavylium form predominates, but at pH 4.5 that the colorless form hemiacetal whish is present. This confirms the results that compared the antioxidant activity and anthocyanins of dried and fresh date's varieties (Baliga *et al.*, 2011).

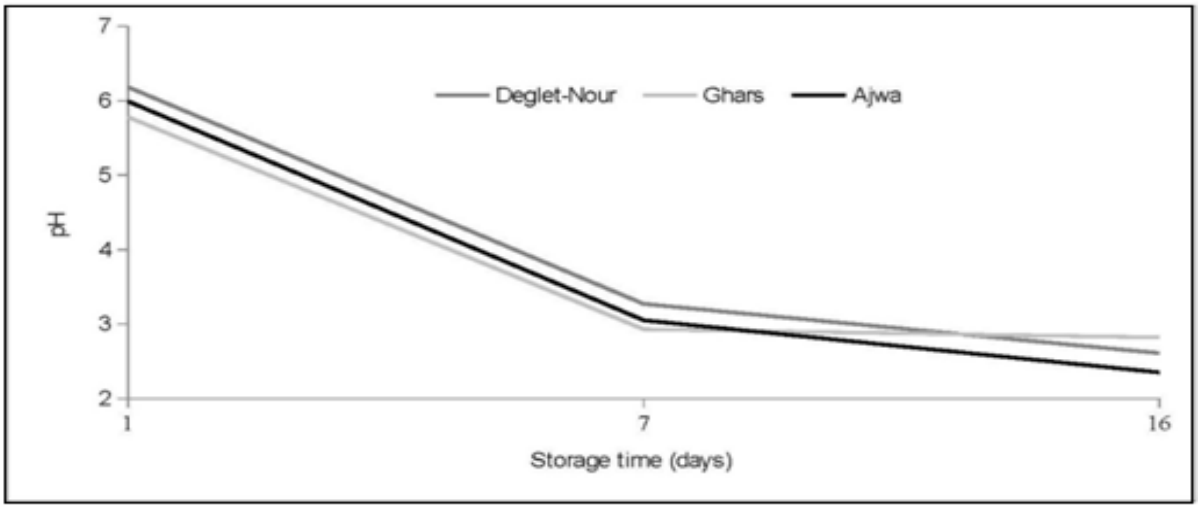

Fig. 3. pH Changes of the Three varieties of dates stored at 22 ˚C during 16 days.

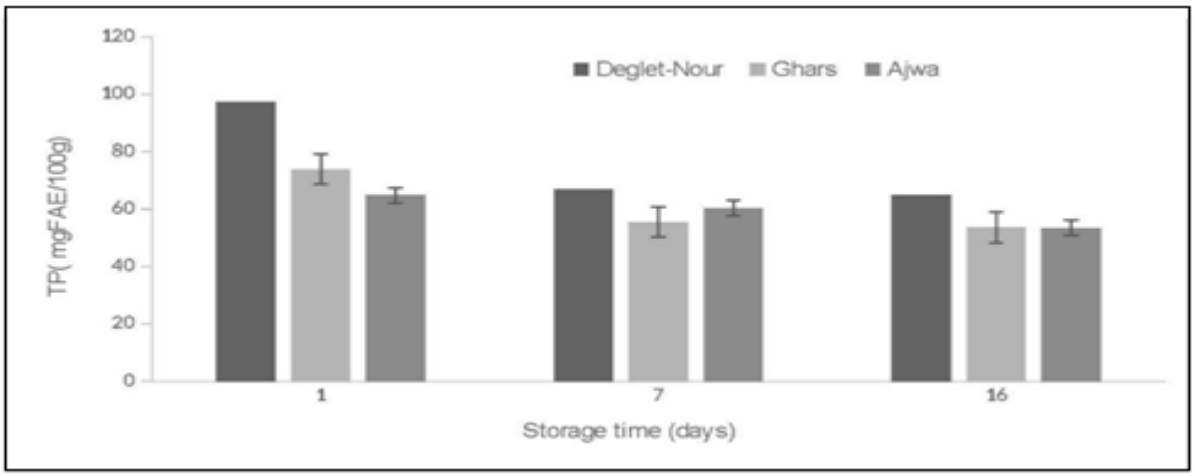

Fig. 4. Total phenolics of three dates varieties water extracted and stored at-18 ˚C within 16 days.

Regarding the antioxidant activity, ORAC-FL used in this study gave highly significant results ($p< 0.05$) for the Deglet-Nour variety followed by the varieties Ajwa and Ghars. In this study, we have tried to compare our results of the variety Deglet-Nour with the other two varieties as there are few studies of the varieties (Ajwa and Ghars) that concern the different parameters studied; antioxidant activity, solvents and anthocyanins. The antioxidant activity of the variety Deglet-Nour was also studied by (Mrabet *et al.*, 2012; Kchaou *et al.*, 2013) using the method of DPPH and they obtained lower values compared to those of the present study. It has been reported by (Tabart *et al.*, 2009; Karasawa *et al.*, 2013; Tang *et al.*, 2013) that the total antioxidant capacity of orange and grape juice by DPPH and FRAP is much lower than by ORAC assay.

Total antioxidant capacity of a food may reflect the combined operations of all antioxidants rather than being a phenolic compound Tabart *et al.* (2010).

The methods ABTS, DPPH,TEAC and FRAP were used by different authors (Biglari *et al.*, 2008; Behija Safi *et al.*, 2009; Awad *et al.*, 2011; Baliga *et al.*, 2011; Hasan *et al.*, 2010; Allaith *et al.*, 2012; Merabet *et al.*, 2012; Kchaou *et al.*, 2013; Benmeddour *et al.*, 2013;Karasawa *et al.*, 2013; Takaedi *et al.*, 2014;Mohamed *et al.*, 2014; Shahdadi *et al.*, 2015) for many varieties of dates and they all reported less values than in our study. Al Asmari *et al.* (2017) have also studied the antioxidant power of some dates varieties using methods as previous authors, and the results were more less for the variety *Sukari* and the variety *Khalas*.

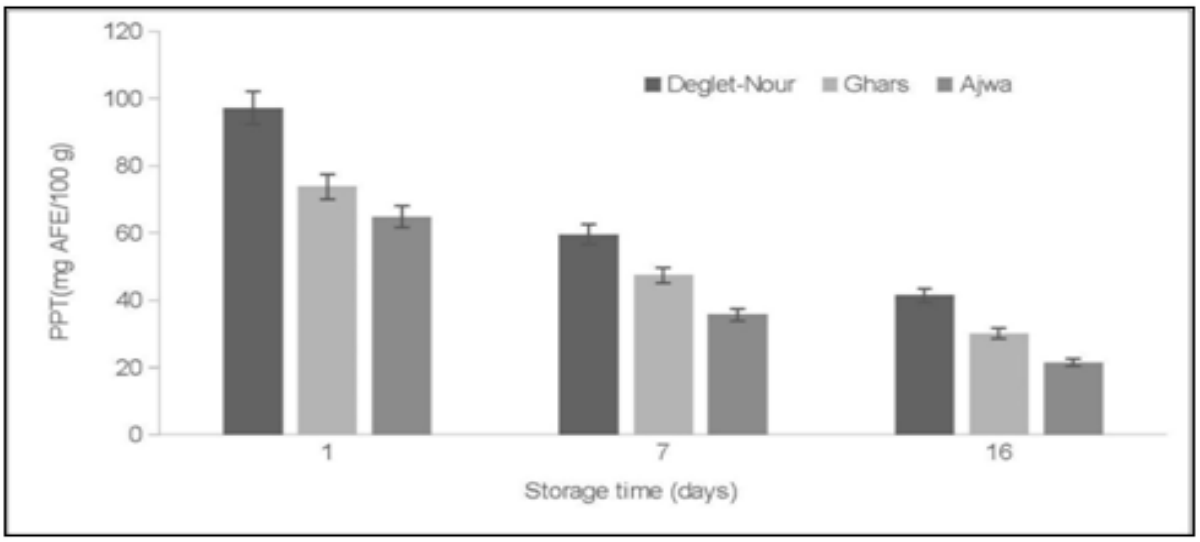

Fig. 5. Total phenolics of three dates varieties water extracted and stored at 22 ˚C during 16 days.

According to Zhang *et al.* (2013), an extract of of Ajwa dates inhibits lipid peroxidation (LPO) by high rate. The treating effect of the extract of dates against the hepatic dysfunction-induced Trichloroacetic (TCA) has been demonstrated by the inhibition of lipid peroxidation of liver.

The improvement of the activity of enzymes superoxide dismutase (SOD) and catalase and improving histopathological changes (Alarem *et al.*, 2014).

pH and total phenolic variation under the effect of temperature and storage time

It was observed a significant effect of the temperature of 22°C and duration of 16 days on the pH of the different varieties of dates studied. We recorded significant variations ($p < 0.05$) from the seventh day of storage until day 16 at a temperature of 22°C.

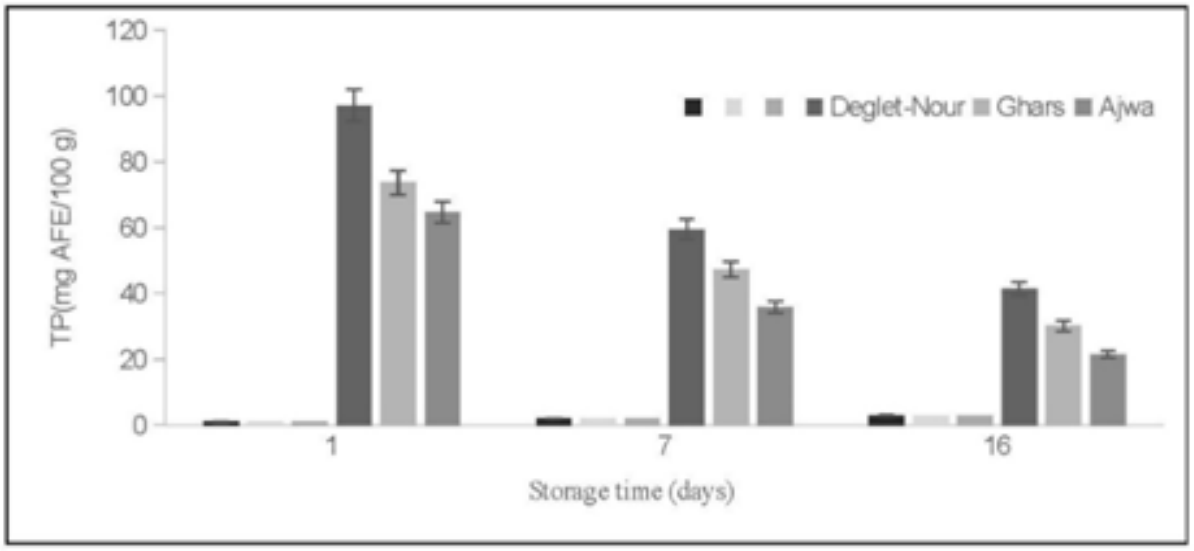

Fig. 6. Total phenolics of three dates varieties (acetone/water) extracted and stored at 18 ˚C within 16 days.

Mohamed *et al.* (2014) have also tested the effect of storage temperature on the total phenolic content of the '*Barhee*" date variety, they observed that the total phenolic of this variety stored at 1 °C decreased from the early hours till the second day.

The storage conditions effect on the physicochemical properties of two varieties of dates "*Khalas*" and "*Barhee*" have shown that these varieties react differently under the same conditions and the best storage conditions studied were two months at -3 °C for *Khalas* variety and one year at -3°C for the "Barhee" variety (Ismail *et al.*, 2008). In light of these results, it is imperative to ensure the sensory and nutritional quality of dates which is largely affected by postharvest treatments and storage conditions.

Conclusion

Our results showed that extraction with aceton/water (70:30) gave higher values compared with hot water at 60°C for the three varieties of dates studied: Deglet-Nour, Ajwa and Ghars. Storage of samples at a temperature of 22°C presented a significant effect on the total phenolic content (a significant reduction) by comparing their preservation at -18 °C or we recorded a slight decrease of these compounds. We noticed a difference in the results for the three varieties of dates studied: Deglet-Nour, Ghars and Ajwa for assays of total polyphenols and anthocyanins. The determination of the antioxidant activity of our samples by the method of the ORAC-FL has given much important results comparing with other studies. The Algerian *Deglet-Nour* variety presented higher rates of polyphenols and anthocyanins and a strong antioxidant activity compared to the varieties *Ajwa* and *Ghars*. Regarding to our results for a better extraction of total polyphenols from dates, the best solvent is aceton alone or combined with other solvents like water, methanol or ethanol. Several studies have reported a positive correlation between the antioxidant activity and the concentration of total polyphenols dates. Whereas in other studies, it was reported that there is no correlation between them. Following this work, we were able to compare different parameters; extraction solvent, Algerian dates varieties stored at different temperatures, their phenolic content, anthocyanins and their antioxidant power. The idea took shape because of few studies that have been conducted for Ghars and Ajwa varieties and to bring new results concerning Deglet-Nourvariety.

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