EVALUATION OF ANTIOXIDANT CAPACITY IN SEVERAL COMMERCIAL VARIETIES OF DATE PALM (*Phoenix dactylifera* L.) FRUITS

Latrach, S.[1]; Verde Rodríguez, A.[2]
e-mail: averde@uvigo.es

[1]Alumna Grado en Biología (Erasmus Mundus)
[2]Alumna Máster en Biotecnología Avanzada
Tutores:
- Mercedes Gallardo **Medina***
- Jesús M. Míguez Miramontes**
Departamento de Biología Vegetal y Ciencias del Suelo
Facultad de Biología
Universidad de Vigo

Abstract

Dates (Phoenix dactylifera, L.) are well recognized as nutrient-rich fruits and also to possess antioxidant properties. The aim of the present work was to study the antioxidant activity (phenolic and flavonoids content, total antioxidant capacity) of six commercial varieties of date palm fruits commercially available in local markets of Vigo (Spain), and to compare it with the levels described in literature for dates obtained directly from producer countries. Results showed that commercial dates are antioxidant-rich fruits since their high content in acid phenolic and flavonoids compounds, which showed values similar to those reported for dates obtained directly in the origin countries. This suggests that antioxidant properties of dates are preserved after fruit industrial processing and commercial distribution.

Introduction

Social and economic importance of dates

Date palms (*Phoenix dactylifera* L.) are considered as one of the oldest fruit crops, which have been cultivated since ancient times in North Africa and the Middle East for at least 5000 years. Date has a long history and a wide distribution that makes difficult to know the exact origin of its cultivation, but it most likely originated from the ancient Mesopotamia (southern Iraq) or western India (Zohary and Hopf, 2000). From its center of origin, date cultivation spread throughout the Arabian Peninsula, North Africa, and the Middle East. The spread of date cultivation later accompanied the expansion of Islam and reached southern Spain and Pakistan. The Spanish were the first to introduce date palms outside of the ancient countries, carrying them to America (Nixon, 1951). Date palm cultivation had a fundamental importance in the history of the Middle East and the north of Africa. Without dates, no large human population could have been supported in the desert regions.

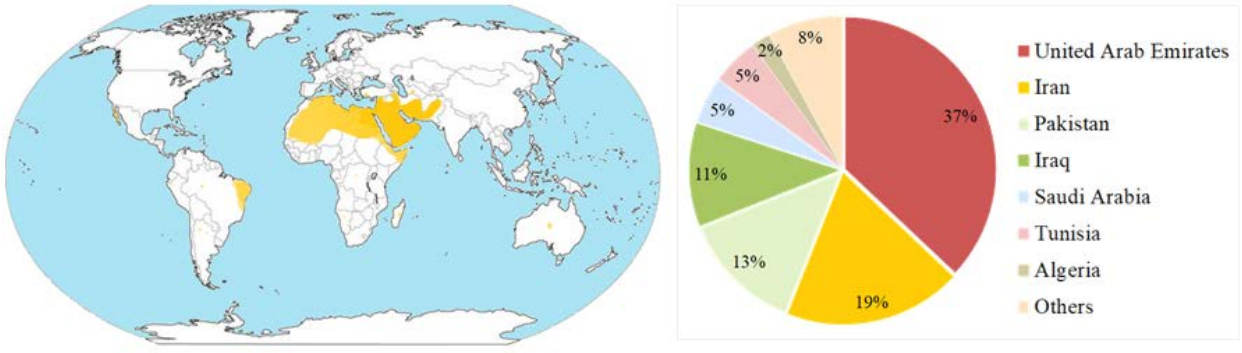

Figure 1. Geographical distribution of date palm in the world (left) and export market (1998-2000) share by region (right). Data obtained from FAO (2002).

Dates are a popular diet complement in many countries in which they are cultivated since its high nutritional value and eligible fruit (Odeh *et al.* 2014). Dates are also a main income source since their important role in agri-food industry that needs to cover the feeding of citizens who includes commonly dates in the diet, but also due to the high product value, which is exported to many countries in the world (FAO, 2002). Figure 1 shows clearly that Middle East and North African countries dominates the export market by far in terms of volume. A further analyses show that North Africa has 26 percent of the market in terms of value, while it represents only 8 percent in terms of quantity. This is a clear reflection of North Africa's strategy to target the high value markets of Europe. Higher export prices are achieved by Israel, Tunisia, United States and Algeria, which have developed a specific production strategy by growing dates varieties of higher quality based on texture, flavor and color.

Depending on the stage of maturity, dates can either be classified as a fruit, comparable to any other fruit consumed between meals or, alternatively, as a food source as part of the daily meal, in particular in the rural areas of the date producing countries. In recent times, there has been a renewed interest in the date as a food source, not necessarily as a staple food, but rather as a component in food preparations like sweets, confectionery, condiments, baking products and health foods (FAO, 1993).

In addition to the nutritional benefits, date fruits have been related to various biological effects including antimutagenic anti-inflammatory, anticancer, antimicrobial and immunostimulant properties mainly due to the high value of antioxidants (Odeh *et al.* 2014).

Date varieties

The date palm accounts for more than 3000 varieties found around the globe. There are about 244 in Morocco, 250 in Tunisia, 370 in Iraq, 400 in Iran, and many additional varieties in the other major date growing countries (Devanand and Chao, 2003; Sedra, 2011). The following are some of the most commonly grown dates:

- **Deglet Nour**: Nour' dates (dates of light in Arabic) are the most widely grown dates in North Africa and the United States. It is amber-colored with a delicate flavor, although not as sweet or moist as other varieties such us the 'Medjoul' date. In addition, 'Deglet Nour' dates are relatively smaller than 'Medjoul' dates. It has firm flesh and a color range from light red to amber.
- **Allighes**: is one of the abundant varieties of Tunisian palms. It has an elongated shape, and a particular delicious taste while looking soft. It is dark with a semi soft quality.
- **Medjbouda**: The Medjbouda dates are a common variety from Tunisia. Their shape is elongated with a jelly brown color. They are medium sized relatively to other dates. Moreover, they have a hard texture and a very sweaty flavor.
- **Kenta**: Kenta variety is originally from Tunisia. It has a light golden color and an attractive aspect, this variety has an early maturity. It is less sweet than the other varieties and has a semi-dry texture.
- **Khadrawy**: Khadrawy dates are medium sweet dates with a dark mahogany color. They have a very soft caramel-like texture that is often compared to the consistency of pudding and sweet flavor. They are similar to the Halawi dates and both are from Palestine.
- **Medjoul**: the 'Medjoul' variety (meaning unknown) is originally from Morocco but largely spread around the world. It is of high commer¬cial value and is considered to be one of the best exported dates with regard to its fruit quality and size in comparison with other varieties. The fruits are considerably large and elongated (5 cm long; 3.2 cm diameter), weighting 20-40 g. At maturity, 'Med¬joul' fruit color is related to the climate and growing conditions but most commonly are of yellow-orange color.
- **Halawi dates**: These soft dates are thick-fleshed, caramel-like texture, and sweet. Their appearance is wrinkled and the skin ranges from yellow to amber.
- **Barhi**: Named for the hot Arabic winds called "Barh," these dates are medium-sized, thin-

skinned fruit with soft, tender flesh and a syrupy flavor.

- **Thoory**: This is a dry date with firm skin and chewy flesh.
- **Zahidi**: The Zahidi, a semisoft date, is called “Nobility.” It has a large seed and crunchy fibrous flesh, and is often processed for sliced dates and date sugar products.
- **Ajwah dates**: Ajwah dates are among the most popular dates in North African and Arabian countries today, since they are a delightfully soft variety with fine texture and sweetness.

Nutritional composition of date fruits

Date fruits are important in human nutrition because of their rich content of essential nutrients, which include carbohydrates, salts and minerals, dietary fiber, vitamins, fatty acids, amino acids and protein. The nutritional value of dates is due to their high sugar content, which is around 50%-75% of dry weight and based mostly of inverted form (glucose and fructose). Fresh varieties contains higher amount of inverted sugars, the semi dried varieties have equal amount of inverted sugars and sucrose, while dried varieties have higher sucrose levels (Nehdi *et al.* 2010; Baliga *et al.* 2011). In addition, dates are fruits rich in mineral elements such as potassium (2.5 times more than bananas), calcium, magnesium and iron, as well as vitamins such as tiamine (vitamin B1), riboflavin (vitamin B2), and niacin (vitamin B3), and also ascorbic acid (vitamin C), pyridoxine (vitamin B6) and folic acid. It contains also bioactive components such as anthocyanins, phenols, carotenoids, and flavonoids, which protect against oxidative stress (Allaith, 2008). Water content of dates is between 7% (dried) and 79% (fresh) depending on variety, cultivar and ripening stage. Dates also represent a good source of vegetable fiber (Borchani *et al.* 2010; Hasnaoui *et al.* 2011).

Dried dates can easily be stored and preserved because of their naturally high sugar content, which change according to the varieties of date fruit and to differences in cultivar, harvest/postharvest factors and growing environment (temperature, humidity), but especially to the maturation stages, which are usually described by the Arabic terms kimri, khalal, rutab and tamer. Many physical changes of date fruit at these stages have been reported (Allaith, 2003), including changes in size and weight, color, texture, astringency and sweetness. The total sugar concentration usually increases from the kimri stage to the tamer stage. As example, Ahmed *et al.* (1995) reported that the total sugar concentration at the kimri stage varied from 3.4% to 7.7%, while that at the tamer stage varied from 44.3 to 64.1%.

Health benefits of dates: An antioxidant-rich fruit

In addition to their significance as an ideal high-energy food, dates are believed to have many medicinal properties. As a consequence, they are used against a number of ailments and pains including fever, stomach disorders, memory disturbances, nervous disorders, as well as to improve the immunity. They are also considered to protect against many chronic diseases including cancer and heart diseases (Duke, 1982), as they have been shown to contain antioxidant and antimutagenic properties (Vayalil, 2002; Al-Farsi et al. 2005; Allaith, 2008).

Antioxidant activity of date fruit has been reported by many investigators (Al-Farsi *et al.* 2005; Amoros *et al.* 2009; Al-Turki *et al.* 2010). Date fruit extract can inhibit protein oxidation as well as neutralize superoxide and hydroxyl radicals. The antioxidant activity is attributed to phytochemical compounds of dates such as phenolic acids, sterols, carotenoids, flavonoids, anthocyanins, etc., as well as the mineral selenium (Abdul Ameer, 2008; Yeh *et al.* 2009). The content of these constituents varies with the fruit variety, the stage of fruit picking, soil conditions and location, with the phytochemicals also contributing to nutritional and organoleptic properties of dates (Abdul Ameer, 2008). Since environmental factors are affecting the antioxidant properties of dates, many studies have been developed in relation with the antioxidant activities of fruits grown in most of the producer countries, including North Africa (Mohamed *et al.* 2014; El Sohaimy et al. 2015), Middle East (Saleh *et al.* 2011; Odeh *et al.* 2014), and the United States (Al-Jasass *et al.* 2015), which also reported the nutritional aspects of date fruits from each geographic location.

The presence of polyphenols and flavonoids in fruit and vegetables usually receive a considerable interest to scientists. These compounds have highly demonstrated antioxidant capacity to act as free radical scavengers and inhibitors of low density lipoproteins, cholesterol oxidation and DNA breakage (Al-Turki et al. 2010). As compared to other fruits, dates can be considered a good source of phenols (Vayalil, 2002; Guo et al. 2003). Thus, Guo *et al.* (2003) reported that dates had the second-highest antioxidant value out of 28 fruits commonly consumed in China, whereas Al-Farsi *et al.* (2005) reported the average contents of total phenolic in fresh and dried dates showing that it was significantly higher in sun-dried dates than in fresh dates. Fresh date Tunisian varieties were also found to be a good source of flavonoids (Chaira et al. 2009), and Hong *et al.* (2006) identified near to 130 flavonoid compounds in date fruit at the khalal stage, whereas Biglari *et al.* (2008) reported that the flavonoid concentration in Iranian date fruit varied according to maturity stages.

Despite the large amount of information available on the antioxidant properties and phenolic compounds of dates from various countries, information regarding the antioxidant potential of commercialized dates commonly found in the European markets is scarce. Many of these fruits do remain for several weeks or months before available for consumers, and even nutritional properties are preserved other functional activities would need to be confirmed to ensure plenty their benefits for human uses. The objective of the present study was to characterize the antioxidant properties of several varieties of date palm fruits that are commercially available in the local markets of Vigo (Spain).

Material and methods

Date palm material

Fruits of six date palm cultivars were obtained from a local supermarket during the months of February and March 2016. Fruits investigated in this study were of the following varieties (cultivar origin is indicated in brackets; (Figure 2): Deglet Nour (Tunisia), Allighes (Tunisia), Medjbouda (Tunisia), Kenta (Tunisia), Khadrawy (Palestine) and Medjoul (Palestine). Sensorial inspection (visual, taste) of fruits suggested that they were collected in a similar maturity stage, although no specific data were available with exception of the market product labelling. Once in the laboratory, date samples (1.0 Kg each variety) were stored at -26 °C for later analysis.

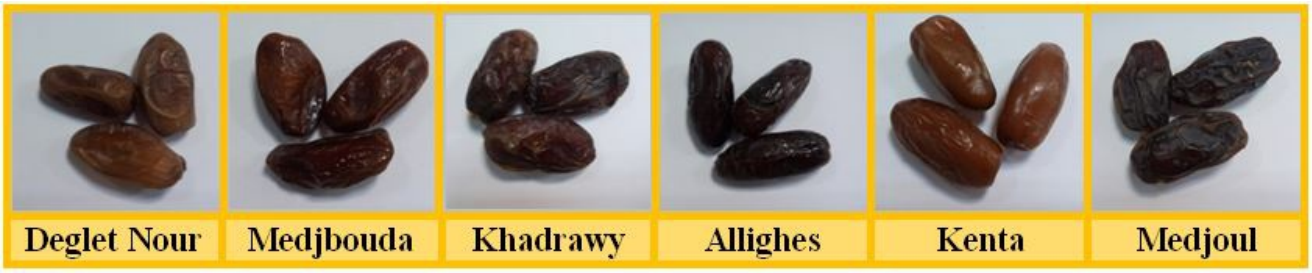

Figure 2. Commercial dates used in this study.

Preparation of date fruit extract

For each extraction, several date palms for each variety were used and four replicates were carried out in parallel during the process. The edible part of date fruits (100 g) was crushed and ground for 5 min using a pestle and mortar. The date palm was then extracted with 300 mL of methanol/water (4:1, v/v) at room temperature for 5 hours using a stirrer. The extracts were then filtered through four layers of cheesecloth and the resulting filtrate was concentrated under reduced pressure at 45°C for 3 hours using a rotary evaporator to obtain the date palm methanol crude extract. The crude extract was kept in dark glass tubes at -26°C until used for analysis.

Antioxidant content of date fruits. Total phenolic and flavonoid content

The total phenolic content was quantified for each date extract according to the method described by Singleton *et al.* (1999) using the Folin–Ciocalteu reagent and gallic acid as a reference standard. Aliquots of 100 µL of each sample prediluted 1/50 or gallic acid standard were tested in quadruplicate, and 0.5 mL of Folin-Ciocalteu reagent (prediluted 10-fold with distilled water) was added to each tube. The tubes were maintained at room temperature for 5 min, afterward, 1.4 mL of 7.5% sodium carbonate (Na_2CO_3) was added and mixed well; then, the samples were incubated for 60 min. The absorbance was measured at 765 nm. The standard curve was prepared with aqueous solutions of known gallic acid concentrations. The method was found to be linear from 2-10 µg/mL with a correlation coefficient r^2 of 0.9968 for the plot of absorbance vs. concentration of gallic acid. Results were expressed as milligram gallic acid equivalents (GAE)/g sample.

The determination of total flavonoids was performed according to the colorimetric assay of Kim et al. (2003). Distilled water (4 mL) was added to 1 mL of date palm fruit extract prediluted 1/10 in a test tube. Then, 0.3 mL of 5% sodium nitrite solution was added. The tubes were maintained at room temperature for 5 min, afterward 0.3 mL of 10% aluminum chloride solution was added. Test tubes were incubated at ambient temperature for 6 min, and then 2 mL of 1 M sodium hydroxide were added to the mixture. Immediately, the volume of reaction mixture was made to 10 mL with distilled water. The mixture was thoroughly mixed using test tube shaker and the absorbance of the pink color developed was determined at 510 nm. The standard curve was prepared with aqueous solutions of known catechin concentrations in the range of 10 - 100 µg/mL (r^2 of 0.9969) and was used for calibration. The results were expressed as mg catechin equivalents (CEQ)/g sample.

Antioxidant capacity by DPPH

DPPH (2,2-diphenyl-1-picryl-hydrazyl-hydrate) free radical method is an antioxidant assay based on electron-transfer that produces a violet solution in ethanol. This free radical, stable at room temperature, is reduced in the presence of an antioxidant molecule, giving rise to colorless ethanol solution. The free radical scavenging activity of the date extract was carried out following the methods of Brand-Williams *et al.* (1995). The stock solution DPPH (24 mg DPPH/100mL methanol) was diluted with methanol to obtain an absorbance of 1.1 at 515 nm. Aliquots of 0.6 mL of the sample extracts, blank (methanol), or Trolox solution as standard was allowed to react with 3 mL of the DPPH working solution for 20 min under dark conditions. Then, the absorbance was read at 515 nm. The standard curve was prepared using Trolox as standard (concentrations in the range of 0-200 µM; r^2 of 0.9947) versus radical scavenging activity and the results were expressed in terms of micromole Trolox Equivalence (TE)/g sample.

Statistics

Data are expressed as mean ± standard error of mean (S.E.M.). The data were subjected to one-way analysis of variance (ANOVA), followed by Student-Newman-Keuls multiple comparison test. Differences between means were defined as significant when P value was lesser than 5% ($p<0.05$). Statistical was performed with Sigma Stat v11.0.

Results and discussion

Dates are well recognized as nutrient-rich fruits and by their sweet, succulent and exotic flavor. Dates have been also shown to possess strong antioxidant activity since their content on phenolic and flavonoid compounds, which are active in neutralization of free radicals and decomposition of peroxides (Guo et al. 2003). The characterization of date fruit for antioxi-dant properties can expand their demand since consumers are increasingly looking for healthy food.

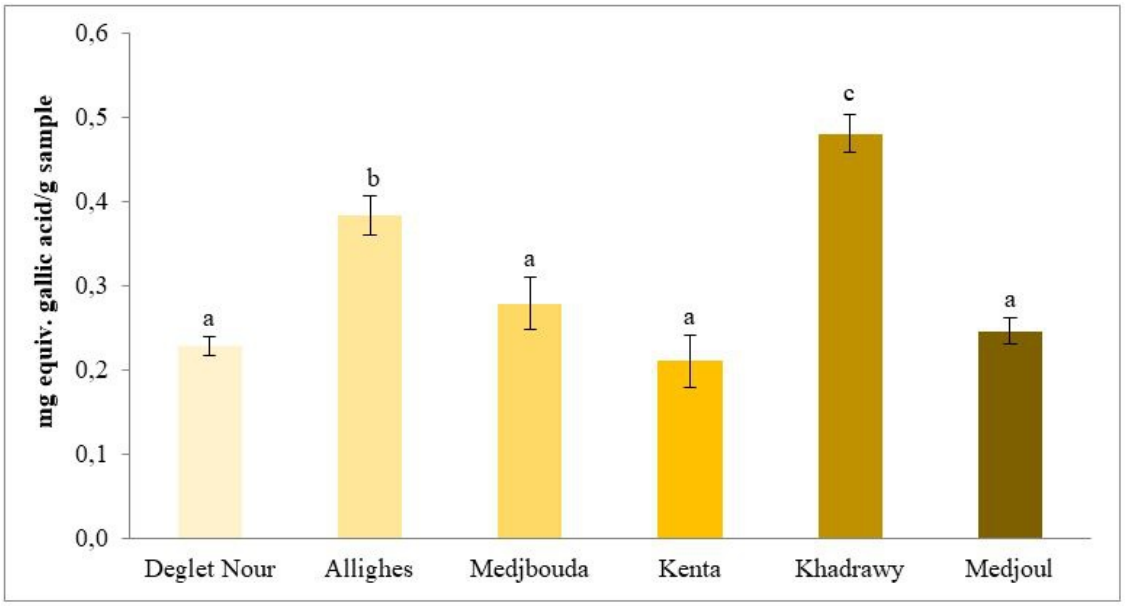

Figure 3. Total phenolic content (mg GAE/g sample) of the different date palm varieties studied. Values are means ± SEM of four samples analyzed. Statistics: Different letters mean significant differences among the respective groups ($p<0.05$).

The quality of dates available for consumers begins from the orchard and ends at the consumer table. Soft dates picked on the tree are still moist and fresh, and at this point they are named as tamar. In the season of tamar harvest some industries receive the fruits in amounts that exceed the markets immediate capacity. Thus, most tamar dates are stored and released into the market according to demand. Since quality parameters are affected by storage and distribution, it is important to understand the effect of such storage conditions on the different characteristics of the date fruit (Ismail *et al.* 2008). To our knowledge, most studies about date properties were carried out on cultivars obtained directly in the producer countries, and only very few ones focused on market available dates coming from foreign countries (Al Jasass *et al.* 2015).

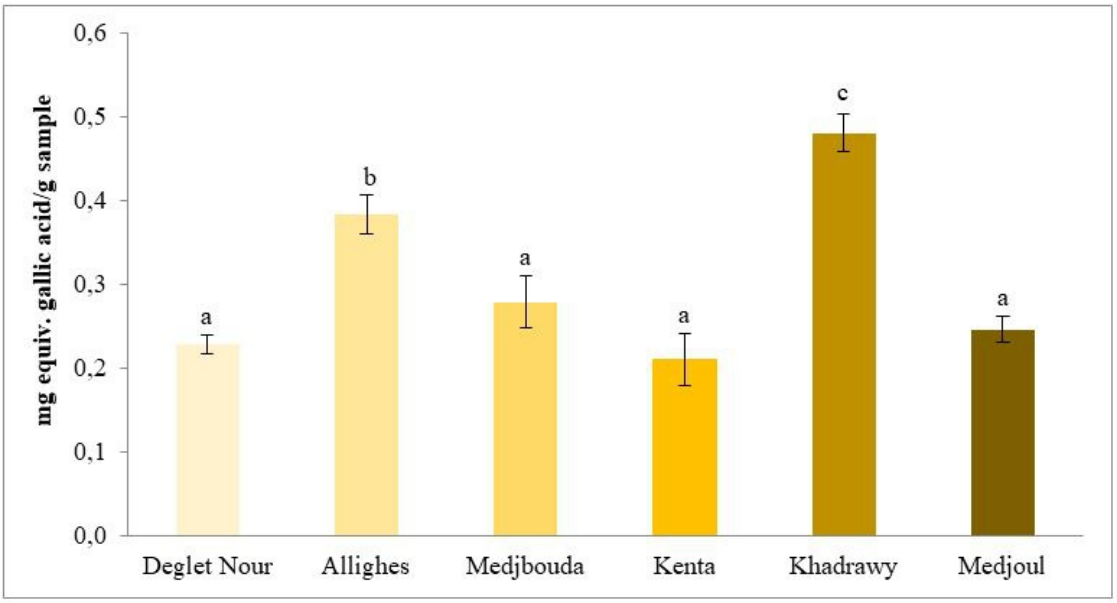

Figure 4. Antioxidant capacity by DPPH (μM TE/g sample) of the different date palm varieties included in the study. Values are means ± SEM of four assayed samples. Statistics: Different letters mean significant differences among the respective groups ($p<0.05$).

In the present study we measured the total antioxidant capacity, and phenolic and flavonoid content in six date palm varieties commercially available in the supermarkets of Vigo (Spain), four of these varieties were coming from Tunisia and the other two from Palestine. Regarding phenolic and flavonoid content, we measured levels from 200 mg GAE/g weight to 450 mg GAE/g weight (Figures 3 and 4), which were in general in the range of date varieties commercially available in the USA (Al Jasass *et al.* 2015), and also agree with date fruits obtained directly from commonly grown cultivars in several North Africa and Middle East countries, including Tunisia (Saafi *et al.* 2009; Kchaou *et al.* 2014), Palestine (Odeh *et al.* 2014), Algeria (Mansouri et al. 2005), and Iran (Biglari *et al.* 2008), among others. However, Al-Farsi *et al.* (2007) found much lower total phenolic contents (ranging from 1720 to 2460 mg GAE/g fresh weight) for Omani dates, whereas Lemine *et al.* (2014) reported Mauritanian dates to have lower phenolic content (about 50-85 mg GAE/g dried weight), which were also affected by ripening. Changes in phenolic and flavonoid content in relation with harvesting date and date palm ripening were also reported in several studies (Saleh et al. 2011; Odeh *et al.* 2014).

Data presented here clearly support that all date fruits at certain maturation stages are rich in the level of phenolic and flavonoid compounds, and also that antioxidant properties are preserved after industrial processing and commercial distribution of dates. In support, total antioxidant capacity measured by the DPPH method (Figure 5) also showed values of anti-radical efficiency for dates that were in a range similar to other studies using different date palm varieties and origin countries (Al-Farsi *et al.* 2005; Mansouri *et al.* 2005). Future studies exploring antioxidant capacity in date palm fruits in different maturation stage would improve our knowledge and will have high scope for application in the valorization of date fruit.

Conclusion

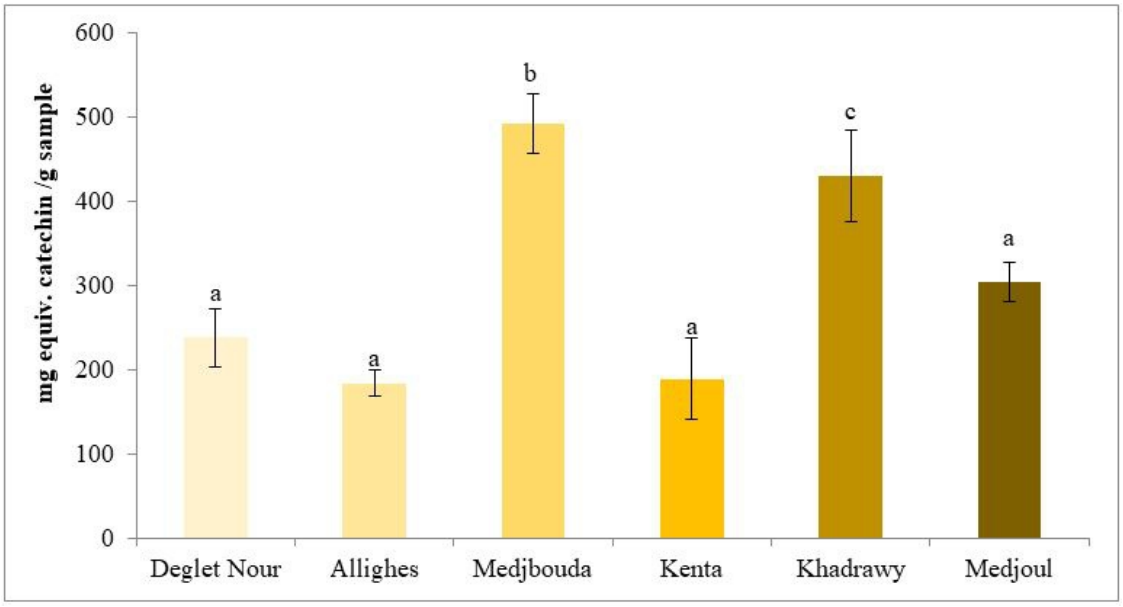

Figure 5. Total flavonoid content (mg CEQ/g sample) of different date palm varieties. Values are means ± SEM of four assayed samples. Statistics: Different letters mean significant differences among the respective groups ($p<0.05$).

Antioxidant activities (phenolic and flavonoid contents, antioxidant capacity) measured in six commercial date varieties available at local markets (Vigo, Spain) were in general similar to those described in the bibliography for dates picked in the origin country. Overall, the six varieties analyzed showed high antioxidant potential that could be important in terms of health benefits for consumers.

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