Title: Date palm Parthenocarpic Fruits (*Phoenix dactylifera* L.): Chemical Characterization, Functional Properties and Antioxidant Capacity in Comparison with Seeded Fruits.

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1. Introduction

Widespread in the worldwide, date palm fruits are gaining popularity for the nutritional and health protective properties. Millions date palm trees are actually cultivated (Hamza et al., 2014) and the date production in the world become important and it has increased significantly in the recent decades. This culture is known from pre-historic time and had been mentioned in several civilizations as an important human food (Elbeker, 1972).

Being dioecious (separate male and female plants), the date palm have an out-crossers model. Many varieties require hand pollination; farmers collect male spathes after their burst and insert male inflorescence into the female one (Honnay and Jacquemyn, 2008). The number of the inserted flowers depends on both the female variety and the pollen quality. Pollination is

better done in a good sunny day as soon as the first break in the female spathe is observed (Hamza et al., 2014).

Despite its tediousness and high cost, hand pollination is strongly recommended especially in the case of Deglet Nour variety in order to ensure the commercial date production (Abd El-Zaher, 2008). During the last decade, many pollination processes have not been done at the best time and under the ideal conditions. This is due to many reasons such as the lack of discipline of workers, climatic changes and the unavailability of sufficient quantity of the best pollen. As a result, the quantity of unpollinated fruits (seedless or parthenocarpic, locally named as Sees) increased which decrease the number of pollinated fruits (economic fruits or seeded fruits) and cause a big economic problem.

Three up to nine months after the ripening of the seeded fruit, the seedless fruits ripen fully. The seedless fruits are smaller and are not as sweet as the seeded fruits (Jaradat, 2014). However, the ripening of seedless fruit could be shortened by cutting bunches at the Besser stage (Khalal stage) and suspended at the ambient temperature during few days suggesting that they could be considered as climacteric fruit.

The aim of the current study was the valorization of unpollinated fruits, both climacteric and naturally maturate ones. In this paper, chemical, and functional characterizations of the seedless fruit were studied in comparison with seeded ones. Any approach about valorization of these under-utilized fruits could be greatly appreciated by producers, whose benefits are being decreasing during the recent years.

2. Materials and methods

2.1. Samples

Dates were collected from “Jemna” oases which belong to continental oases, situated in Southern Tunisia. This area was chosen for its production which is the highest in comparison

with the other continental oases. Three localities were studied: Sanya (9.008992E 33.575301N), Kouchada (9.011449E 33.548905N) and Om Errouss (8.984885E 33.553573N) coded respectively as “S”, “K” and “O”. These oases are on deep sandy soils, poorly fertile with a coarse texture and rich in gypsum. The climatology parameters are as follow: the mean temperature is 21.4°C, the relative humidity is 54.3% and the annual precipitation is about 65mm.

A set of 900 seeded fruits and 900 seedless ones were collected from the Deglet nour variety. Seeded fruit were picked at commercial maturity (Tamer stage: fully-ripe stage) during the harvest season of 2013 at the beginning of December. Seedless fruits were collected during two different times. The first was in December 2013: bunches that contain unpollinated fruits at Besser stage were suspended inside the farm and kept in ambient temperature for five days to reach the Tamer stage. The second was in March, 2014, and the collection was at the full natural maturation stage. All dates were stored in a refrigerator at approximately 4°C for analysis.

2.2. Chemical composition

For the humidity, up to 10 g of the flesh fruit was freeze-dried and the moisture content was determined by the difference in weight between before and after the assay and expressed as a percent. Both Ethanol soluble fraction (ESF) and the ethanol insoluble residue (EIR) was prepared by homogenizing a quantity of 20g of flesh dates in 250 ml of ethanol (80%) and then filtrated. Soluble sugars were determined from ESF according to the anthrone method. Neutral sugars, uronic acids, proteins, and Klason lignin were determined in EIR as described previously (Fuentes-Alventosa et al., 2009). For neutral sugars (NS), EIR was hydrolyzed with 72% sulfuric acid at 40°C for 2 h, and 6% sulfuric acid at 100°C for 2 h. The released sugars were quantified as alditol acetates by gas chromatography. A HP 6890 Plus+ gas chromatograph (Hewlett-Packard, Palo Alto, CA, USA) fitted with a 30 m × 250 µm × 0.20

mm capillary column (SP-2330, Supelco, Bellefonte, PA, USA) was used. The carrier gas was helium with a constant flow = 2.2 mL/min and pressure = 21.5 psi (148.24 kPa). Injection was performed in splitless mode. The oven temperature was held at 50 °C for 2 min after injection, then programmed to 180°C at 35°C/min, held at 180°C for 5 min, and then immediately increased to 220°C at 5°C/min and held at 220°C for 22 min. Total run was 40.7 min. The injector temperature was 250°C and flame ionization detector, 300°C. Myo-inositol was used as internal standard. Uronic acids were quantified using the phenyl–phenol method after sulfuric acid hydrolysis (Ahmed and Labavitch, 1977). Klason lignin levels were determined as the amount of acid-insoluble material remaining after a sulfuric acid hydrolysis. Proteins were analyzed according to the Kjeldahl method using a Büchi Digestion Unit, K-424, and a Büchi Distillation Unit, K-314, and applying a factor of 6.25 to convert the total nitrogen into protein content.

2.3. Water and oil Holding Capacities (WHC and OHC)

WHC and OHC were determined using the method described by Jiménez et al. (2000). Samples (250 mg × 3) of EIR were suspended in 15 mL of water or sunflower oil (1.0054 g/mL density). After 24 h of stirring at room temperature, the suspension was centrifuged at 14000g for 1 h. Supernatants were carefully eliminated, and the hydrated or the oil-embedded fibers were weighted. WHC was expressed as milliliters of water per gram of EIR. Hydrated pellets were freeze-dried, and their solubility in water was determined by the difference of weight before and after the WHC assay, which was expressed as a percent. OHC was expressed as milliliters of oil per gram of EIR.

2.4. Determination of Soluble Phenols

Total polyphenol content was quantified for each date ESF according to the Folin–Ciocalteu spectrophotometric method, using gallic acid as a reference standard. Aliquots of 20 µl of each liquid fraction were dosified in triplicate, and 100 µL of Folin–Ciocalteu phenol reagent

(0.2 M) was added to each tube and mixed. Afterward, 80 μ l of Na_2CO_3 (75 g/L) was added and mixed well. A microplate reader (iMark, BioRad) was set at 655 nm, and the absorbance was measured after 6 min. Results are expressed as gallic acid (GA) equivalents (mg/100 g FW).

2.5. Determination of the Antiradical Activity

Soluble antioxidant activity was determined from the ESF by the DPPH $^{\bullet}$ method (Rodríguez et al., 2005). Ten μ l of the ESF was added to 190 μ l of DPPH $^{\bullet}$ (3.8 mg/50 mL methanol), after 30 min in obscurity the absorbance was measured (in triplicate) at 480 nm. EIR antioxidant activity was evaluated as described by Fuentes-Alventosa et al. (2009). Between 3 and 14 mg of EIR was transferred to an eppendorf tube (all EIRs were diluted with cellulose as an inert material), and the reaction was started by adding 1.5 mL of the DPPH $^{\bullet}$ reagent. After 30 min of continuous stirring, samples were centrifuged and the absorbance of the cleared supernatants was measured (in triplicate) at 480 nm. Both antioxidant activities were expressed as millimoles of Trolox equivalent antioxidant capacity per kilogram of the sample by means of a dose-response curve for Trolox.

2.6. Ferric Reducing Power

A modification of the Psarra et al. (2002) method was used. The FeCl_3 was employed as an oxidant. The ion Fe^{2+} produced from the redox reaction forms a colored product with 2,2'-dipyridyl. For the determination of the reducing power of extracts, a microplate reader was used. Ten μ L of each ESF, standard, and their dilutions and 10 μ L of 6 mM FeCl_3 in 5 mM citric acid were placed in each microplate well in quadruplicate. In each sample, a blank without FeCl_3 was included. The microplate was incubated during 20 min at 50°C. Following this, 180 μ L of 5 g/L dipyridyl solution in 1.2% trichloroacetic acid was added. After 30 min the absorbance was measured at 490 nm. The ferric reducing power was expressed as

millimols of Trolox equivalent antioxidant capacity per kilogram of sample by means of a dose-response curve for Trolox.

2.7. Statistical Analysis

Results are expressed as the mean value $\pm$ standard deviation. The studied parameters were analyzed separately by ANOVA with post-hoc SNK comparisons. The statistical program was SPSS 12.0 for Windows Release 12.0.0 (4 Sep. 2003, SPSS Inc.).

3. Results

3.1. Chemical composition

The climacteric seedless fruits showed a significant increase of the moisture (Table 1), the mean value was 13.14%. The mean humidity in the seeded and the natural seedless fruits was 10.53% and 10.73%, respectively. Significant difference was observed between localities in the case of seeded fruit and natural seedless fruits. Soluble sugars measured in ESF varied between 55.85 and 68.14g/100g FW for the seeded fruit with a mean of 60.91g/100g FW. However, in climacteric seedless fruit the values ranged between 48.97 and 57.80g/100g FW with a mean of 53.32g/100g FW. Natural seedless fruit have the smallest quantity of sugar in comparison with the others types of fruits (Table 1). Within the three localities and in natural seedless fruits, the same amount of soluble sugars was detected but significant differences were underscored in the case of seeded and climacteric seedless fruits.

Uronic acids values ranged from 11.82 to 20.54 g/100g EIR (Table 1). The highest mean values were for natural seedless fruits with a mean of 18.30 g/100g EIR. However, seeded and climacteric seedless fruits had lower quantities with a mean of 13.62 and 12.77 g/100g EIR, respectively. No significant effect of the locality was observed in all types of the studied fruits. Conversely to uronic acids values, natural seedless fruits have demonstrated the lowest lignin content with a mean of 30.30 g/100g EIR. Lignin contents of seeded and climacteric

seedless fruit were higher and the means was 37.92 and 34.39 g/100g EIR, respectively. As the uronic contents, lignin ones were not affected by localities.

Protein content was between 7.63 and 8.94g/100g EIR (Table 1). Climacteric seedless fruit protein content was higher than the other types of the studied fruits but this difference was not significant. Significant locality effect was observed only in natural seedless fruits.

The neutral sugar (NS) contents of the studied fruits are also reported in the Table 1. NS were the most abundant component after lignin, ranging from 14.07 to 25.22g/100g EIR (1.5-3.3 g/100g FW). The origin of the samples did not lead to significant differences. Seeded and climacteric seedless fruits had the same level, about 16 g/100g EIR, but natural seedless ones had NS content much higher, near 24 g/100g EIR. The NS composition is presented in percentage of total insoluble sugars in Figure 1. The main recorded sugar was glucose with a mean of 55-60%. Comparing the three different samples, seeded and climacteric seedless fruits showed significant differences in all the sugars, but between seeded and natural seedless ones there were significant differences only in xylose and galactose.

3.2. Water and Oil Holding Capacities

The WHC of seeded date palm fruit was ranged between 6.64 and 9.42 mL/g EIR (Table 2). Concerning climacteric seedless fruits WHC was between 7.89 and 8.29 mL/g EIR and for natural seedless fruit the rates were between 7.31 and 9.91 mL/g EIR. No significant differences were observed between localities and between the three types of fruits. For the solubility, non-statistical difference was observed between types of fruits and the rates ranged between 0.20 and 0.37%.

The OHC was between 3.46 to 4.41 g oil/g EIR for seeded fruits and between 3.70 and 4.11 g oil/g EIR for natural seedless ones. Climacteric seedless fruits were the highest with 4.40-5.62 g oil/g EIR. Moreover, localities had little effect on the OHC values of the studied fruits, only

Kouchada locality for both seeded and climacteric seedless fruits showed significant differences.

3.3.Soluble Phenols and Antioxidant Capacity

The results for soluble phenols and the antioxidant capacity of the ESF and EIR are presented in Table 3. Natural seedless date palm fruits had the highest soluble polyphenol content (573.26 mg GA/100 g FW) with a significant effect for locality. However, low values were found in seeded and climacteric seedless fruits and no locality effect was observed. Climacteric seedless fruit did not show significant differences with seeded fruits in phenol content (Table 3).

Antioxidant activity of date palm fruits was analyzed in this study as DPPH antiradical efficiency and as ferric reduction power and the results are presented in Table 3. ESF antiradical activity was higher in the case of natural seedless fruit in comparison with the others. Locality had not a significant effect on antiradical activity of seeded fruit and of climacteric seedless fruit but was significant for natural seedless fruits. A high correlation was observed between phenol content and antiradical activity with a value of $R^2=0.9851$.

Table 3 shows also the values of ferric reducing power of ESF of the studied samples. The samples had the same gradation found in DPPH antiradical activity, natural seedless fruit power were higher than other dates power. Moreover, we have also found a high correlation between phenol content and ferric power reduction ($R^2=0.9239$). The antiradical activity of EIR was also measured (Table 3). In almost all samples, no significant differences were caused by sample origin. In seeded and climacteric seedless dates, soluble activity was lower than that of the EIR (expressed in fresh weight basis, mmol Trolox/Kg FW), as it is observed in Table 3.

4. Discussion

Concerning the moisture, similar results were obtained by other investigations on seeded fruits of the same variety collected in the season of 1996 and 2006 (Bouabidi et al., 1996; Mrabet et al., 2012). It was proved that the ripening process decreases the fruit moisture (Booij et al., 1992). In this work, seeded and natural seedless fruits were collected at the full maturing process and the climacteric seedless ones at Besser stage. The reach of climacteric seedless to Tamer stage was accelerated, which explains the higher moisture in these fruits than in seeded ones. In addition, oases effect was observed and could be attributed to differences in irrigations techniques and / or humidity and structural variations of the soils (Al-Juburi et al., 1994; Al-Hooti et al., 1997).

Different sugar levels have been found by various authors. Chaira et al. (2007) found a content of soluble sugars of 72.82 g/100g FW, Elleuch et al. (2008) reported a quantity of 79.1 g/100g FW, for Besbes et al., (2009) work 87.55 g/100g FW, El Arem et al. (2011) an amount of 63.16 g/100g FW and recently Jemni et al. (2014) have found 79.58 g of total sugars in 100g FW. Sugar concentrations varied with ripening stage, firmness and water content (Jemni et al., 2014). The high sugar content in the last maturation stage (Tamer) is due to the decrease of moisture (Al-Shahib and Marshall, 2002). Soluble sugars in climacteric seedless were lower than the seeded ones which could be due to the method of material collection. The decrease of this parameter is very important for consumers who like Deglet Nour variety with a smaller amount of sugar. The smallest quantity of sugar was in natural seedless fruit which could attribute to the date palm seed an important role in sugar accumulation in the fruit during the natural maturation process.

For seeded fruit, lignin content was lower than those underscored by Mrabet et al. (2012) which found a quantity of 50.37% of date dietary fiber. These authors found also a lower quantity of uronic acids in dietary fiber extracted from Deglet Nour fruit variety. These differences could be due to the different composition of date pulp and date dietary fiber.

Elleuch et al. (2008) found lower quantities of lignin and uronic acids. The consumer's acceptability could be influenced by the lignin and pectin content of date fruits; good edible fruits have low lignin and high pectin levels (Shafiei et al., 2010). Hence, from this point of view, natural seedless fruits seemed to be better than climacteric seedless and seeded fruits, which had similar characteristics. The founded protein content is in accordance with other results reported for the same variety. In fact, USDA (2007) has noted a quantity of 2.45 g/100g of Deglet Nour fresh material. Other investigations have demonstrated a protein content between 2.3 and 5.6 g/100g FW in Saoudian cultivars and between 2 and 2.5 g/100g FW in Emartian ones (Al-Hooti et al., 1997).

The glucose amount corresponded to a content of cellulose between 0.9-2.0 g/100g FW and this was in agreement with other publications (Mrabet et al., 2012; 2015), where the cellulose content of several date varieties were studied and ranged from 0.99 to 1.62 g/100g FW. The rest of sugars, mainly coming from hemicelluloses and pectins, were in much lower amounts being the relative amounts in agreement with those previously reported (Mrabet et al., 2015). Seeded and natural seedless date fruits have very similar composition in their polysaccharides, having the second ones a higher amount than the former. In the contrary, seeded and climacteric seedless fruits have the same amount of polysaccharides but with different composition. When these results are expressed as g/100g FW (data not shown), the locality did not show a significant effect on neutral sugar composition for the seeded fruits. For the climacteric seedless fruits, only galactose and glucose were affected by locality. However, all neutral sugars were affected by the fruit origin in the case of natural seedless fruits. Natural seedless date fruits seemed to be a good source of dietary polysaccharides as pectins, hemicelluloses, and cellulose.

WHC is an important property of foods and food ingredients from both physiological and technological points of view. WHC values were lower than those obtained in previous

investigations on Deglet Nour dietary fiber (Mrabet et al. 2012), probably due to the difference in composition and structure of both starting materials. These WHCs are similar to banana peel fiber concentrate (Wachirasiri et al., 2009) and apple and pear pomace (Rabetafika et al., 2014). Alison and Cummings (1979) expressed that water uptake is severely related to uronic acids content. Natural seedless fruits have shown a significant higher amount of uronic acids that could imply an increase in WHC, although it was not significant. OHC for seeded fruits was similar to previously observed on date palm dietary fiber (Borchani et al., 2010; Mrabet et al. 2012). In this study, climacteric seedless fruits showed higher OHC than other fruits which suggest that seedless material could be also used in the formulation of new products with good emulsifying properties. Daou and Zhang (2011) studied the relationship between the emulsifying capacity and the protein level in the fiber fractions derived from rice bran. They observed that higher amounts of proteins correspond to higher capacity. Analogically, climacteric seedless fruits have the highest protein content which could enhance their oil holding capacity.

There are many parameters that affect the phenol content in date palm fruit. Biglari et al. (2009) reported that there is a significant correlation between fruit consistency and phenol content in Iranian dates which is confirmed by other investigations (Al-Farsi et al., 2007; Mansouri et al., 2005). The method of phenol extraction and storage duration of fresh material can also affect the quantified amount of soluble phenol (Jemni et al., 2014; Mrabet et al., 2012). Muntaha and Batool (2011) have demonstrated that phenol content in date palm fruit cv. Hillawi decreased during the fruit ripening, the lowest content was at the last stage (Tamer). However, these same authors working with parthenocarpic fruits from the same variety observed that their content in tannins were much higher than that of normal fruits and that they remained in the fruits in significant amounts even at the 29th week after pollination. Tannins are responsible for the astringency of immature date fruits, and make them inedible.

Many works have evaluated the date soluble antiradical activity and have shown that Deglet Nour's one was the highest in comparison with other varieties (Jemni et al., 2014; Mrabet et al., 2012). In the current study, antiradical activity of natural seedless fruit was the highest compared with other dates.

The antioxidant activity of the residue could be very important with regard to the total antioxidant activity of fruits and vegetables because, as Saura-Calixto (2011) suggested, the transportation of antioxidants through the gastrointestinal tract is an essential physiological function of dietary fiber. However, this aspect has received very little attention so far. Expressed as dry weight (mmol Trolox/kg EIR), the antiradical activity was higher than other Tunisian varieties (Garen Gaze, Eguwa, and Smeti) already published (Mrabet et al., 2015). Although in this case the difference among samples was lower than in other characteristics, natural seedless fruits reached again the highest values, the soluble one was about 8-9 times higher than the residue. This kind of date fruits could be an excellent source of soluble phenols.

5. Conclusion

According to these results, we can conclude that the seedless parthenocarpic Deglet Nour fruits could be valorized due to their chemical, functional and technological characteristics. In spite of the different shape of seeded and climacteric seedless fruits, both samples were chemically and functionally very similar, and the climacteric ones could be considered as edible fruits, although their content in soluble sugar was a bit lower than normal fruits. The application of the climacteric maturation process of these fruit could be a technological resolution of this problem which enhances the conservation of the major proprieties and accelerates its harvest. Natural seedless date fruits had some characteristics that make them inedible due to their astringency but suitable for industrial applications. They are very rich in phenolics and insoluble polysaccharides (pectins, hemicelluloses, and cellulose) which are the

main components of dietary fiber. They could be considered as an excellent source of soluble phenols and dietary fiber. A simple extraction with low graduation ethanol solution could extract soluble sugars and phenolics, which are easily purified by adsorption chromatography. In this way, three different interesting fractions could be obtained from these unused fruits: the first one, rich in soluble sugar, useful for confectionery and patisserie; the second one, a phenolic concentrate with very high antioxidant activity; and the third one, a solid residue interesting as dietary fiber source. This technological approach for natural seedless fruits must be carried out in order to change this agricultural residue into valuable byproducts.

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