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Chemical Composition of the Flesh and the Pit of Date Palm Fruit and Radical Scavenging Activity of Their Extracts

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Abstract: The chemical analysis of flesh and pit of two varieties of date palm fruit (Deglet nour and Alig) as well as the radical scavenging activity of their extracts was undertaken. The fruits were grown in Djerid region (Oasis of Tozeur, Tunisia). Total sugars was measured by HPLC method or with Fehling reagent, crude lipid content was obtained by Soxhlet's extraction, mineral elements were measured by atomic absorption photometer. The antioxidant activity of extracts from the flesh and pit of the date fruit was measured on the basis of the scavenging activity of the stable 1, 1-diphenyl-2-picrylhy-drazyl (DPPH) free radical. Our results showed that the total sugars could present over 60% of the dry weight of date flesh in two studied varieties. The content of reducing sugars was higher in Alig variety than Deglet nour. For the minerals content, dates flesh were a very good source of several minerals and could be an important source of potassium for regular consumer with a concentration of 0.61 and 0.72% (dry matter), respectively from flesh Deglet nour and Alig. Concerning the date pit composition, oils content was 10.13% in Deglet nour and 12.37% in Alig and the total sugars were less than 6% for the two varieties. In addition, ethyl acetate extracts from flesh Deglet nour, pit Deglet nour and pit Alig showed an important free radical scavenging activity towards 1-1-diphenyl-2-picrylhydrazyl (DPPH) free radical.

Key words: Date palm, oasis, composition, flesh, pit, DPPH

INTRODUCTION

The date palm (*Phoenix dactylifera* L.) is considered the most important source of food for both human in arid and semi-arid regions. Dates contain a high percentage of sugars reaching 88% in some varieties (Al Shahib and Marshall, 2003). Dates are also rich in mineral salts and vitamins (Booij *et al*., 1992). For the date pit, the percentage of non-reducing sugars is 3.82% and in glucose and fructose is 1.68 and 1.53%, respectively (Fayadh and Al-Showiman, 1990).

Lately, several therapeutic virtues are assigned to the date palm and its derivatives. Extracts of the dates provided to the women after childbirth stimulate their immune system (Puri *et al*., 2000). On the other hand, a polysaccharide isolated from dates presented an antitumor activity (Ishurd and Kennedy, 2005). Extracts of the pits date decrease quickly and meaningfully the women's wrinkles (Bauza *et al*., 2002). In addition, it has been found that date pits would make a profit for date farmers. In the Middle East, for instance date pits are used in animal feed (Al Dabib, 2005; Hamada *et al*., 2002). Several investigations one the effect s of pits in animal diets suggest the presence of substantial amounts of tannins and resistant starch and natural anabolic agents (El-Gasim *et al*., 1995). Until now, insufficient data are available on the radical scavenging activities of date flesh and date pit palm. In fact, metabolic action of carcinogen is a free radical-dependent reaction (Freig *et al*., 1994; Guyton and Kensler, 1993) and many synthetic antioxidants are used as antioxidants. However, food like fruits and vegetables rich in natural antioxidants were used to reduce risks of inflammation and problems caused by cell coetaneous aging (Bauza *et al*., 2002; Ames *et al*., 1993). The antioxidant activity of these foods can be attributed for their antioxidants compounds, such as ascorbic acid, vitamin E, carotenoids and selenium (Fain, 2004; Guyton and Kensler, 1993), but also, flavonoids, tannins and other phenolic constituents (Salah *et al*., 1995; Van Acker *et al*., 1996). The objectives of this study were to evaluate nutritional quality of flesh and pit from two varieties of date and to make a comparative study of radical scavenging activity of their extracts.

MATERIALS AND METHODS

Chemicals reagents: Methanol, Fehling's A Reagent DC and Fehling's B Reagent DC were purchased from Panreac

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Quimica SA (Barcelona, Spain). Analytical Reagent grad Ethyl Acetate and Acetonitrile HPLC grad were obtained from Lab-Scan (Dublin, Ireland). Sucrose standard and 1, 1-diphenyl-2-picrylhy-drazyl were purchased from Fluka (Buchs, Switzerland). Glucose, fructose standards and vitamin E were purchased from Carlo Erba (Val de Reuil, French). Absolute ethanol was from Sigma Aldrich (Seelze, Germany) and petroleum ether (40-60°C) was from Lab-Scan (Dublin, Ireland). The water used in HPLC and sampling was prepared with Millipore Simplicity (Millipore S.A.S, Molsheim, French).

Date palm cultivars: Dates palm fruits (Deglet nour and Alig) were collected in December 2005 at tamr stage from the oasis of Tozeur. In fact, the Alig variety presents 23.22% palms plantations. Alig has a medium appearance, a pale brown color, a semi dry to dry consistency and a late maturity. It is about a late and good conservation date, which permits to farmers to consume it a long time after harvest. It mainly used in making pastry date. The samples of two varieties were collected, at stage of complete maturity (tamr stage), from some palms of Tozeur oasis (30 palms variety^{-1}) nearly 600 fruits per variety.

The dates were conserved in ice, transported to laboratory and stored at -20°C. The analyses were carried out in January 2006 in Aridlands and Oases Cropping Laboratory of the institute of the arid areas of Medenine (Tunisia). The seeds were isolated, washed and both flesh and seeds were further air dried at 50°C and then milled to pass powdered flesh and pit.

Analytical methods

Water content of date flesh and date seed: Water content of date flesh and date seed was determined according to the method of Official Analytical Chemists (AOAC, 1990).

Ash and minerals contents: To remove carbon, about 1 g (powdered flesh or pit) in a porcelain container, was ignited and incinerated in the muffle furnace at about 530°C for 5 h. Ashes were expressed as percent of dry weight. The mineral constituents (Ca, Na, K, Mg, Cu, Zn, Fe and Mn) present in the date flesh and pit were analyzed separately, using an atomic absorption photometer (Shimadzu A 6800). The samples were prepared for analyses as described by Al Showiman (1990). Phosphorus content (P) was determined by the spectrophotometer (Secomam 1000, French).

Sugars content

Sugars content of date flesh

Extraction and sample preparation: The samples were prepared for analyses as described by Booij *et al.* (1992). Each sample was filtered over a 0.45 μm membrane filter and analyzed by Liquid Chromatography.

Liquid chromatography conditions: LC separation was carried out at room temperature on Eurospher NH2 column, 100 A pore size, 7 μ mm particle size, 250 mm, 4.6 mm I.D (Knauer, Germany). Prior to use, solvents were filtered over a 0.45 μm membrane filter and sonicated for 15 min in a Ultrasonic Cleaner Model SM 25E-MT (Branson Ultrasonics Corporation, Dambury, USA). The mobile phase used was acetonitrile-water ultra-pure (80%: 20%, v v^{-1}). The LC was connected to RI Detector K-2301 from Knauer (Germany). The flow-rate and the injection volume during the experiment were 1.0 mL min^{-1} and 20 μL, respectively. The integrator was calibrated with external standards consisting of solutions of glucose (2%), fructose (2%) and sucrose (1%). Total reducing sugars were obtained as the sum of glucose and fructose values. Each sample was analyzed in triplicate and quantification was carried out from integrated pick areas of the sample against the corresponding standard graph.

Sugars content of date pit: A total sugars as well as reducing and non reducing sugars was measured by Fehling reagent.

Lipids content: About 20 g of powdered flesh and pit were used. Lipid extraction was carried out by Soxhlet extractor with 250 mL of petroleum ether.

Radical scavenging activity on DPPH: The cartridge was put in a Soxhlet apparatus (250 mL). Extracts from each vegetal material were prepared using successive solvents of increasing polarity (chloroform, ethyl acetate and methanol). After removing solvent, using Rotavapor apparatus, three types of extracts of different polarities were obtained from each vegetal:

- Extracts of chloroform from Deglet nour flesh (ECFDn), Deglet nour pit (ECPDn), Alig flesh (ECFA) and Alig pit (ECPA),
- Extracts of ethyl acetate from Deglet nour flesh (EAEFDn), Deglet nour pit (EAEPDn), Alig flesh (EAEFA) and Alig pit (EAEPA).
- Extracts of methanol from Deglet nour flesh (EMFDn), Deglet nour pit (EMPDn), Alig flesh (EMFA) and Alig pit (EMPA).

To ensure their stability, these extracts were conserved under the stream of nitrogen and stored at least -18°C.

The free radical scavenging capacity of the compounds test was determined with 1, 1-diphenyl-2-picrylhy-drazyl (DPPH). An aliquot of each tested extracts at various concentrations (100, 30, 10, 3 or 1 mg mL^{-1} in ethanol) were mixed with 23.6 μg mL^{-1} of DPPH solution (in ethanol) (v v^{-1}). After incubation of the mixture for

30 min, the absorbance of the remaining DPPH was determined calorimetrically at 517 nm. The scavenging activities were expressed as a percentage of absorbance of control DPPH solution (Yagi *et al*., 2002) and vitamin E was used as control positive. Antioxidant activity was calculated using the equation:

$$\% \text{ antioxidant activity} = 100 * [1 - (As / Ac)].$$

With As is the absorbance of sample and Ac is the absorbance of control sample.

Each sample was analyzed in triplicate and results were expressed as percentage activities.

Statistical methods: All analytical determinations were performed at least in triplicate. Values of different parameters were expressed as the mean±standard deviation.

RESULTS AND DISCUSSION

Chemical composition of date flesh and date pit: Table 1 presents the average chemical composition of date flesh and date pit of two studied varieties. Date flesh from Deglet nour and Alig contained 21.12 and 26.68% moisture, respectively. These results were in agreement with those found by Booij *et al*. (1992) who mentioned the amount of 25% for Deglet nour. Works of Ahmed and Ahmed (1995) showed that date moisture can vary between 9.2 to 32.1%. In opposition, this content of water could reach highest values in others fruits, this concentration was 53.72% in strawberry (Ozcan and Haciseferogullari, 2007), 84.57% in pomegranate (Al-Maiman and Ahmad, 2002) and 86.89% in apple (Martin-Esparza *et al*., 2006). Indeed, this weak content in moisture of studied varieties permits a better conservation and inhibits the development of bacteria.

The ash content in Deglet nour and Alig flesh was 1.3 and 1.1%, respectively. This in agreement with previous reports (Booij *et al*., 1992; Reynes *et al*., 1994). Concerning the crude lipid content, this value was ranging from 0.22% for Alig variety to 0.28% for Deglet Nour, however, Al-Hooti *et al*. (1998) found a higher content (0.5%) in dates flesh from United Arab Emirates varieties.

The total sugars content in Deglet nour flesh was 72.82% of dry matter and a comparable result was found by Reynes *et al*. (1994). This tenor could not exceed 14.1% in pomegranate (Al-Maiman and Ahmad, 2002), 21% in pineapple (Grizotto *et al*., 2007) and 6.25% in blackberry (Kafkas *et al*., 2006). Concerning the Alig variety, this content was 60%. The relative sucrose amount was 53% in Deglet nour flesh but only 5% in Alig. In fact, the practical subdivision of dates in soft, semi dry and dry is correlated to increasing sucrose content. Our results confirmed this hypothesis because the Alig variety was classified as soft date and Deglet Nour exceptionally as semi dry date (Reynes *et al*., 1994). Alig flesh showed a higher reducing sugars content (55.0% of dry matter) than Deglet Nour flesh (17.7%). In fact, the reducing sugars are the important component, since they immediately bring some energizing calories available (Vaandercook *et al*., 1979).

For the two studied varieties, the minerals concentration of date flesh was near to the average nutrient intake (Table 2). The most important mineral was potassium followed by calcium, phosphorus, magnesium, sodium, iron, zinc, copper and manganese. These results agree those reported by Reynes *et al*. (1994). Calcium and phosphorus, which are often in deficit in foods, have been found in relatively important quantity in these two varieties of dates. The ratio calcium/phosphorus (1.1 for Deglet nour and 1.2 for Alig) permits a good nutritional balance. It was also obvious that date flesh was rich in trace elements and modern nutritional research confirmed

Table 1: Chemical composition (dry basis) of date flesh and date pits from Deglet Nour and Alig (Tunisia)

Component	Date variety	Dry matter (%)	Fat	Reducing sugars	Non reducing sugars	Total sugars	Ash
			(% dry matter)				
Flesh	Deglet nour	79.880±0.384	0.283±0.014	17.737±0.326	55.083±0.144	72.820± 0.250	1.303±0.014
	Alig	73.327±0.094	0.227±0.005	55.197±0.571	5.090±0.033	60.287±0.595	1.107±0.014
Pit	Deglet nour	86.877±0.116	10.130±0.033	2.180±0.152	3.473±0.311	5.653±0.187	1.170±0.056
	Alig	87.977±0.116	12.737±0.110	2.287±0.074	3.157±0.044	5.443±0.054	1.107±0.005

Table 2: Mineral content of date flesh and date pits from Deglet Nour and Alig (Tunisia)

Component	Date variety	Potassium	Magnesium	Calcium	Phosphorus	Sodium	Zinc	Copper	Iron	Manganese
		(% dry matter)				mg 100 g^{-1} dry matter				
Flesh	Deglet nour	0.619±0.016	0.052±0.001	0.073±0.001	0.065±0.001	13.233±0.233	2.467±0.053	2.167±0.107	6.933±0.053	1.130±0.018
	Alig	0.722±0.008	0.038±0.001	0.052±0.002	0.043 ±0.002	9.100±0.092	2.433±0.053	2.167±0.053	4.300±0.185	1.083 ±0.071
Pit	Deglet nour	0.238±0.001	0.048±0.001	0.034±0.001	0.058±0.001	9.573±0.056	1.177±0.046	1.040±0.009	1.887±0.088	0.353±0.035
	Alig	0.289 ±0.001	0.048 ±0.001	0.026±0.001	0.070 ±0.001	10.370±0.081	1.363±0.044	1.123±0.023	1.763±0.027	0.273±0.019
Nutrient intake (g/day) (Anonymous, 1991)		-	0.300	0.700	0.550	1.600	0.0095	0.0012	0.0087	0.0046

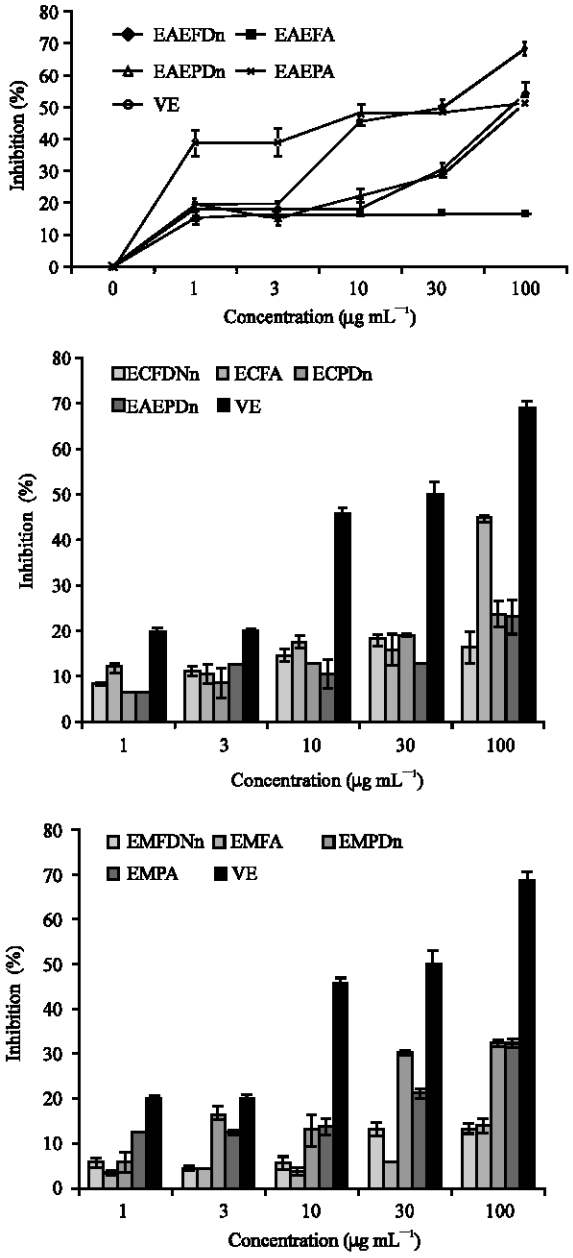

Fig. 1: Antioxidant activities on scavenging the DPPH free radical of extracts from date flesh and date pit

that trace elements are of vital importance to the life activities of the human body. The participation of zinc, iron and copper in many kinds of enzymic composition might play an important role in promoting the metabolism of organisms to strengthen immune ability and the control of disease (He, 1998).

For date pits, the moisture content of Deglet nour and Alig were 13.13 and 12.3%, respectively. The ash and fat content in Deglet Nour and Alig were 1.17, 1.10, 10.13 and 12.73 %, respectively (Table 1). The total sugars in Deglet Nour and Alig were 5.65 and 5.44% and these results were less than those found by Fayadh and Al-Showiman (1990) (7%). Concerning the mineral content, the potassium tenor was the highest, followed in decreasing order by phosphorus, magnesium, calcium, sodium, iron, zinc, copper and manganese (Table 2). However, Al-Showiman *et al.* (1990) found that the calcium content is highly significant, followed by potassium, sodium and magnesium.

Radical scavenging activity on DPPH: Besides anthocyanins, other flavonoids, phenolic acids and vitamins like ascorbic acid and vitamin E can contribute to the protective effect against oxidative damage to cells. Since the antioxidant activity of individual dietary compounds cannot always be evaluated, the determination of the total antioxidant activity allows a more realistic evaluation of the potential protective effect of a food.

The radical scavenging activities of the compounds measured as decolorizing activity following the trapping of the unpaired electron of the DPPH are shown in Fig. 1. In fact, only vitamin E and ethyl acetate extracts presents a potent radical scavenging. The decrease percentage of the absorbance of DPPH standard solution was 68, 54, 51 and 51%, respectively for vitamin E, ethyl acetate extract from flesh Deglet nour (EAEFDn), pit Alig (EAEPA) and pit Deglet nour (EAEPDn) at a concentration of 100 µg mL^{-1}. EAEPA has the strongest DPPH radical scavenging activity. It required only a concentration of 29 µg mL^{-1} to scavenge 50% of free radical followed by EAEFDn and EAEPDn.

Indeed, chemical study of tested extracts, revealed the presence of important quantities of flavonoids and tannins in ethyl acetate extract and recent studies have shown that these flavonoids and related polyphenols contribute significantly the total antioxidant activity of any fruits and vegetables (Luo *et al.*, 2002).

CONCLUSIONS

Dates are known for their nutritional value. They are rich in salts and vitamins but their sugar tenors make them a first class food. In addition, the date flesh has potential in various aspects of the human biological such controlling diseases and enhancing metabolism of the human body.

For the antioxidant activity, a comparison was made with reference compound (E vitamin). The radical scavenging activity of the ethyl acetate extracts from flesh of Deglet nour, pit of Alig and pit of Deglet nour approached the activity of the standard. This information shows that these ethyl acetate extracts can be used as antioxidant in food and medicine preparation.

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