Antioxidant activity, volatile and nonvolatile composition of two Phoenix dactylifera L. by-products

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ARTICLE INFO

Article history:
Received 14 Apr 2017
Received in revised form 9 May 2017
Accepted 12 Jun 2017
Available online 16 Jun 2017

Keywords:
Date palm fruit
Seeds
Physicochemical composition
Aroma composition
Total phenolic content
In vitro antioxidant activity

ABSTRACT

Objective: To examine the nonvolatile components of the date pulp and seeds of two minor Tunisian date palm cultivars Arechti and Korkobbi at besser stage and to investigate their antioxidant activity, their phytochemical compounds and their aromatic volatiles composition.

Methods: The physico-chemical properties, the aroma composition, the mineral profile, the phytochemical content and the in vitro antioxidant activity of those two common date palm fruit varieties and those of their seeds were evaluated. All measurements were made at the besser stage, which showed the highest amount of secondary metabolites.

Results: New volatiles compounds are detected in both flesh and seeds and the results revealed that those by products are a suitable source of mineral content and have a high potential of natural antioxidants and good antiradical capacities.

Conclusions: Thanks to their characteristics, they could potentially be considered as a cheap resource for new functional food ingredient and novel pharmaceutical applications.

1. Introduction

The date palm is the symbol of the Saharan life. It has had for a long time an important place in the world especially in the Middle East and North Africa. There are more than 2000 varieties of date palm in the world and more than 250 varieties in Tunisia[1]. The most known one is Deglet Nour, which accounts for over 50% of the total palms cultivated in Tunisia. This fast evolution of the Deglet Nour variety caused the loss of a considerable percentage of other varieties, which are considered of lower quality but constitute about 30% of the production[2].

Previous works showed that these varieties should be considered as good sources of many useful nutritional and healthy ingredients. Previous reports mentioned that some of these varieties are rich sources of dietary fiber, which could be added in foods[2]. Other studies proved that it contains even better constituents than Deglet Nour such as vitamin C and minerals[3]. Besides many Tunisian minor cultivars have remarkable antioxidant content, which could be useful in food and medicinal preparations[4].

On the other hand, large mass of date seeds, accounting for almost 30% of the manufacture in Tunisia, is produced as a waste of many date processing plants. Many researches showed that date seeds contain a significant amount of bioactive phenols which may explain its potential positive health properties, such as antiinflammatory, antimicrobial and antioxidant activities[5,6].

Despite the enhanced benefits of common date varieties and the good content of phytochemical compounds of their seeds, many Tunisian cultivars are menaced by extinction by their traditional use. Each plant is characterized by its own volatile compounds. According to the literature, a few studies have illustrated the volatile compounds of some dates varieties at mature stage (Stage Tamr). In this context, more than 150 different compounds have been identified[1,7].

Generally, there are four ripening stages for the date fruits. Several studies reported that dates contain the highest amounts of these...
chemicals at the besser stage[8].

Therefore, this study aims to evaluate the nutritional values (proximate and mineral compositions) and the volatile profil of the date pulps and seeds of two minor Tunisian date palm cultivars at besser stage and to investigate their antioxidant activity and their phytochemical compounds.

2. Materials and methods

2.1. Standards and reagents

2,2-Diphenyl-1-picrylhydrazyl (DPPH), 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) diammonium salt (ABTS), gallic acid and Folin-Ciocalteu’ s reagent were purchased from Sigma–Aldrich Co. (St. Louis, France).

2.2. Samples

Two cultivars of date palm (Phoenix dactylifera L.) fruits, Korkobbi and Arechti, were collected in Gabes (Southern Tunisia), during the 2013 harvest season, at besser stage. The flesh part was crushed to obtain a homogeneous paste and a portion of the seed mixture, after being washed and air dried, was crushed. The moisture content was determined (2 g of each sample in a drying oven at 80 °C for 24 h). The seeds remaining part was dried at 50 °C and ground into fine powder.

2.3. Nutrition value

The pH levels, the ash, total soluble protein and fat contents were determined as described previously[7]. The total sugars contents were determined using the phenol sulfuric method[9] and the dinitrosalicylic acid method was used to measure reducing sugars[10].

For the minerals composition, a sample of 1 g was incinerated for 24 h at 550 °C. The residue of incineration was extracted using the method described by Jelled et al.[11]. The mineral compounds were measured using inductively coupled plasma optical emission spectrometry.

2.4. Volatile sampling and analysis

The volatile compounds were determined using SPME coupled by GC-MS system. Quantitative comparisons of relative peaks areas were performed between the same chemicals in the different samples. The identification of the constituents was based on a comparison of the retention times with those of authentic samples, comparing their linear retention indices, against commercial (NIST 2000 and Adams 2007) and home-made library mass spectra and MS literature data[12].

2.5. Phytochemical characterization

Each sample (1 g) was extracted twice by stirring with 30 mL of methanol. Methanolic extracts were redissolved in methanol (final concentration 5 mg/mL) for antioxidant activity evaluation.

The total phenolic content (TPC) was determined using a colorimetric assay described by Jelled et al.[13] based on the reduction of the Folin-Ciocalteu reagent by the samples and expressed as mg of gallic acid equivalents (GAE)/g extract.

Total flavonoid content (TFC) and condensed tannins content (CTC) were determined as described previously[11]. (+)-Catechin was used as standard and the results were expressed as mg of (+)-catechin equivalents (CE)/g extract.

For anthocyanins content, samples were macerated in acidified methanolic solvent [HCl (3 mol/L): H2O: MeOH 1:3:6], under continuous stirring for 24 h. The mixture was incubated for 24 h at 4 °C. The absorbance of the extract is measured at 530 nm and 657 nm[13].

The anthocyanins content was calculated using the following formula and expressed as µg/100 g fresh weight:

\[
\text{Anthocyanins} = (\text{DO}530) - 0.33(\text{DO}657)
\]

2.6. Antioxidant activity

Various concentrations of methanolic date pulp or pits extracts (0.3 mL) were mixed with 2.7 mL of methanolic solution containing DPPH radicals (6 × 10−5 mol/L). The antioxidant activity was measured using an improved ABTS method. 3.9 mL of ABTS+ solution was added to 0.1 mL of the test sample and mixed vigorously, incubated for 6 min and read the absorbance at 734 nm.

For the reducing power, the test samples were mixed with sodium phosphate buffer (pH 6.6) and 1% potassium ferricyanide. After incubation at 50 °C for 20 min, 10% trichloracetic acid was added and the mixture was centrifuged. A total of 5 mL was mixed with deionised water and 0.1% ferric chloride, and read at 700 nm.

The extract concentration providing 0.5 of absorbance (EC50) was calculated from the graph of absorbance against extract concentration. Trolox was used as standard[13].

2.7. Statistical analysis

SPSS 18.0 (SPSS Inc., Chicago, IL, USA) was used to perform the statistical analysis. Data were subjected to One-way analysis of variance for means of comparison, and significant differences were calculated according to Duncan’s multiple range test at P < 0.05. Data are reported as means ± SD. Correlation analysis was performed employing Pearson’s test.

3. Results

3.1. Chemical composition of date pulp and pits

The average compositions of the date flesh (pulp) and pits of the two minor date cultivars Arechti and Korkobbi are presented in Table 1. Moisture, total sugar and reducing sugar contents were much higher in date pulps while date pits exhibited the highest amount of soluble protein and fat contents.

Table 2 presents the mineral compositions in the four samples. Date fruit contained significant amounts of minerals. In the flesh, potassium was the highest among macro-elements (673.00–830.00 mg/100 g dry weight). Whereas, iron was the highest as micro-element (1.16–1.92 mg/100 g dry weight). The zinc was negligible.
Table 1
pH level and chemical compositions of two cultivars of date palm fruits and their pits at besser stage.

| Component   | Moisture | Total sugar* | Reducing sugar* | Protein* | Lipid* | pH  | Ash*   |
|-------------|----------|--------------|-----------------|----------|--------|-----|--------|
| Pulp Arechti (91%) | 31.89 ± 0.16 | 40.02 ± 0.50 | 34.66 ± 0.20 | 3.42 ± 0.63 | 0.48 ± 0.06 | 6.65 ± 0.26 | 1.03 ± 0.01 |
| Pulp Korkobbi (89%) | 36.49 ± 0.46 | 39.62 ± 0.90 | 24.37 ± 0.40 | 2.26 ± 0.63 | 0.36 ± 0.40 | 6.48 ± 0.07 | 1.09 ± 0.04 |
| Pits Arechti (9%) | 5.72 ± 0.15 | 7.60 ± 0.90 | 4.43 ± 0.80 | 5.30 ± 0.50 | 9.02 ± 0.04 | 6.17 ± 0.06 | 1.08 ± 0.20 |
| Pits Korkobbi (11%) | 9.17 ± 0.10 | 9.48 ± 0.18 | 5.33 ± 0.20 | 5.84 ± 0.45 | 8.93 ± 0.20 | 6.61 ± 0.60 | 1.06 ± 0.02 |

Data expressed as means ± SD of three independent extractions (n = 3). Means in each column followed by different letter indicate significant differences (P < 0.05). * Data are expressed in g per 100 g of fresh weight basis; † Data are expressed in g per 100 g of dry weight basis.

Table 2
Mineral profile of two cultivars of date palm fruits and their pits at besser stage (mg/100 g dry weight).

| Minerals      | Sodium (Na) | Potassium (K) | Calcium (Ca) | Iron (Fe) | Copper (Cu) | Magnesium (Mg) | Manganese (Mn) | Zinc (Zn) |
|---------------|-------------|---------------|--------------|-----------|-------------|----------------|----------------|----------|
| KP            | 43.60 ± 0.14| 391.00 ± 0.10 | 47.80 ± 0.08 | 8.40 ± 0.10 | 2.31 ± 0.03 | 93.20 ± 0.31 | 0.47 ± 0.00 |< 0.015 |
| AF            | 18.20 ± 0.09| 830.00 ± 0.37 | 180.00 ± 0.30 | 1.92 ± 0.05 | 1.72 ± 0.02 | 62.10 ± 0.14 | 0.29 ± 0.05 |< 0.015 |
| AP            | 45.80 ± 0.12| 322.00 ± 0.15 | 30.40 ± 0.05 | 5.05 ± 0.08 | 1.52 ± 0.05 | 74.60 ± 0.20 | 0.85 ± 0.08 |< 0.015 |
| KP            | 21.20 ± 0.08| 673.00 ± 0.03 | 60.00 ± 0.07 | 1.16 ± 0.02 | 0.46 ± 0.00 | 62.50 ± 0.11 | 0.17 ± 0.01 |< 0.015 |

Data expressed as means ± SD of three independent extractions (n = 3). Means in each column followed by different letter indicate significant differences (P < 0.05). AF: Arechti fruit; KF: Korkobbi fruit; AP: Arechti pits; KP: Korkobbi pits.

(0.015 mg/100 g dry weight). In the seed, potassium was the predominant macro-element (322.00–391.00 mg/100 g dry weight). For the micro-elements, iron was the predominant one (5.05–8.40 mg/100 g dry weight), and the zinc was negligible.

2.3. Volatile compounds

The results of volatile compositions are reported in Table 3. Plants produce a range of volatile compounds which are mainly comprised of esters, alcohols, ketones, lactones, terpenoids and apocarotenoids. A total of 68 volatile compounds which accounted for 94.9%–99.7% of the total aroma were detected, namely 8 esters, 4 alcohols, 47 terpenes, 3 aldehydes, 3 ketones, 1 hydrocarbons, 2 phenols and 1 phenylpropanoid. In the Arechti pulp, Korkobbi pulp, Arechti seed and Korkobbi seed 35, 28, 35 and 37 compounds were detected as odor-active compounds, respectively. Only nine of the 68 identified compounds (camphene, α-phellandrene, 1,8-cineole, camphor, α-copaene, ar-curcumene, α-zingiberene, β-bisabolene, β-sesquiphellandrene) were detected in all the four samples. Esters and alcohols were the main chemical classes detected in Korkobbi pulp (67.6% and 20.2%, respectively). In the case of Arechti pulp, the sample exhibited the highest amount of terpenes (78.7%). The seeds of the two date varieties showed totally different emission profiles of volatile compounds (Figure 1). The Arechti and Korkobbi seeds (pits) were characterized by a high amount of terpenes (59.1% and 57.9%, respectively).

![Figure 1](https://via.placeholder.com/150)

Figure 1. Proportion of the different chemical classes of volatile compounds of the pulp of two cultivars of date palm and their seeds at besser stage.
3.3. Non-volatile compounds

Table 3 presents the phytochemical compounds in the four samples. Korkobbi pulp had an important amount of total phenols (2940.00 mg/100 g extract) and an appreciable level of flavonoids, condensed tannins and anthocyanins (791.00 mg/100 g extract, 2940.00 mg/100 g extract) and an appreciable level of flavonoids, samples. Korkobbi pulp had an important amount of total phenols

| Table 4 | Phytochemical contents of the methanolic extracts of two cultivars of date palm fruits and their pits at besser stage. |
|---------|-------------------------------------------------------------------------------------------------|
| Samples | TPC | TFC | CTC | TAC |
| AF      | 2940.00 ± 2.98 | 791.00 ± 0.91 | 458.00 ± 0.59 | 1786.00 ± 0.03 |
| KF      | 568.00 ± 0.15  | 164.00 ± 0.00  | 149.00 ± 0.00  | 726.00 ± 0.11  |
| AP      | 2410.00 ± 0.86 | 3399.00 ± 1.81 | 2013.00 ± 0.39 | 2479.00 ± 0.17 |
| KP      | 4431.00 ± 0.07 | 2921.00 ± 1.16 | 2681.00 ± 0.96 | 3290.00 ± 0.80 |

Data expressed as means ± SD of three independent extractions (n = 3). Means in each column followed by different letter indicate significant differences (P < 0.05). TAC: Total anthocyanins content (µg/100 g fresh weight); AF: Arechti fruit; KF: Korkobbi fruit; AP: Arechti pits; KP: Korkobbi pits.

3.4. Antioxidant activity

The EC₅₀ values determined by DPPH assay, ABTS assay and ferric reducing antioxidant power of the four samples differed significantly (P < 0.05) (Table 5). The antioxidant activity determined in seeds is higher than flesh (lower EC₅₀ values indicate a higher antioxidant activity).

A significant correlation (Table 6) is registered between polyphenols content and the antioxidant activity, which seems closely related to these ubiquitous plant compounds.

| Table 5 | Antioxidant activity (EC₅₀, mg/mL) of methanolic extracts obtained from two cultivars of date palm fruits and their pits at besser stage. |
|---------|------------------------------------------------------------------------------------------------|
| Cultivars | DPPH | ABTS | Reducing power |
| AF      | 1.55 ± 0.199a | 1.32 ± 0.36a | 0.65 ± 0.00a |
| KF      | 4.62 ± 0.12b | 2.14 ± 0.19b | 1.08 ± 0.03b |
| AP      | 0.74 ± 0.16c | 0.40 ± 0.12c | 0.27 ± 0.01c |
| KP      | 0.61 ± 0.01d | 0.52 ± 0.02d | 0.25 ± 0.01d |

Data expressed as means ± SD of three independent extractions (n = 3). Means in each column followed by different letter indicate significant differences (P < 0.05). AF: Arechti fruit; KF: Korkobbi fruit; AP: Arechti pits; KP: Korkobbi pits. EC₅₀: Effective concentration 50%.

| Table 6 | Pearson’ correlation analysis between the antioxidant activity and phytochemical composition of two common date palm fruits and pits at besser stage. |
|---------|-------------------------------------------------------------------------------------------------|
| TPC | DPPH | CTC | TAC |
| DPPH | 0.987 | -0.832 | -0.794 |
| ABTS | -0.982 | -0.964 | -0.909 |
| Reducing power | -0.983 | -0.938 | -0.901 |

*: Significant correlation for P < 0.01. TAC: Total anthocyanins content.

4. Discussion

For the physicochemical composition, the pH values are in good agreement with those reported for the three Tunisian cultivars Beidh Hmam, Rfb and Khalet Ahmar at besser stage (pH values ranging between 5.2 and 6.55)[7] and with those measured by Kulkarni et al.[14] for immature date (pH = 6.3).

The moisture content of dates decreases according to the ripening stage. It has the highest level at kimiri stage and the lowest one at tamar stage[7]. Also these values confirmed the previous studies; in fact, in comparison with the data reported by Mrabet et al.[3],
the moisture content of the two cultivars Korkobbi and Arechti at besser stage was higher than that reported at tamar stage.

The dates are not a rich source of protein, but they contain more protein than other fruits[15]. It ranges between 5.5% at kimiri stage and 2.5% at tamar stage. The level of soluble protein was in agreement with those found by Awad et al.[16] for ‘Lonet Mesaed’ and ‘Helali’ cultivars. On the contrary, the percentage of proteins was not in agreement with the values published by Rastegar et al.[17] for Shahani, Piarom and Deiry cultivars; these results could be explained by the different type and origin of cultivars and/or the measuring procedure.

In dates flesh, lipids are mainly concentrated in the skin, where they reach low values ranging from 0.1% to 0.9%[15]. Our results in dates flesh, lipids are mainly concentrated in the skin, where they reach low values ranging from 0.1% to 0.9%[15]. Our results were characterized by low percentage of moisture (3.1%–12.5%) and proteins (5.0%–12.5%) and proteins (5.0%–12.5%) were similar to those of El Arem et al.[7], who reported a fat content comprised between 0.2% and 0.5% but they are in contrast with those of Mohamed et al.[18] for Sudanese date palms which ranged between 1.71% and 2.0%. These differences could be explained by a different maturation stage and/or cultivar types. The same was true for the ash content of the two cultivars Korkobbi and Arechti.

Several studies reported that the sugar content increases when the fruit moves from khallal to tamar stage. They account for about 20% of dry matter at kimiri stage, increasing to 50% dry matter at the beginning of Khalal and reaching 72% to 88% of dry matter at tamar stage[19]. Mrabet et al.[3] verified that at tamar stage Korkobbi dates contain the highest level of total and reducing sugar in comparison with the others Tunisian littoral palm cultivars. It contains more than 50% of total sugar and 39% to 46% of reducing sugar. Our study confirmed the previous results, which are in agreement with those of El Arem et al.[7].

In comparison with the pulp, it was noted that date palm seeds were characterized by low percentage of moisture (3.1%–12.5%) [6] and total sugar (2.2%–8.17%)[10,20]. On the contrary, they contained an appreciable amount of fat (5.0%–12.5%) and proteins (2.3%–6.9%)[6]. In our study, the total sugar levels were higher than those reported for Alig and Deglet nouri seeds (5.44%–5.65%) [21].

Those differences may be attributable to the different studied cultivars and/or to the different climatic and local conditions.

Concerning the mineral composition, our results are in great compatibility with those of many others studies which showed that dates fruits and seeds are a suitable source of minerals[15,17,22], Thanks to its richness in minerals, the dates have a beneficial effect on human health. In addition, several minerals such as calcium, potassium and magnesium proved their ability to reduce cardiovascular disease mortality, blood and bone related disorders[23,24].

Our study showed that the two fruits pulp had a clear difference in their emission profiles concerning proportions and kind of volatile compounds. In contrast, the two seeds shared the same kind and almost had the same proportion of terpenes. Although different plants often share the same qualitative volatile composition, each plant has its own aroma, depending upon the specific characteristics of its individual volatile compounds.

For example, myrcene has an almost citrusy aroma and sweet balsamic herbaceous taste at low concentration, while at higher concentration its notes become more pungent and bitter[25].

According to the literature, a few studies have illustrated the volatile compounds of some dates varieties at mature stage (stage Tamr). In this context, more than 150 different compounds have been identified[1,7,26-29]. As far as we know, with the respect of the study of El Arem et al.[7], which report on Tunisian dates at three maturation stages, including besser one, this is the first endeavor to determine the aromatic compounds of dates at besser stage. Of the components identified at this stage of maturation, 17 compounds have not been previously reported in the literature and are specific for this two Tunisian date palm fruits: 1 alcohols (2-undecanol), 1 phenol (p-vinylnuaciacol) and 15 terpenes (among which β-sesquiphellandrene, β-bisabolene, α-zingiberene and ar-curcumene were the main ones).

The number and the kind of volatile compounds detected in our study are quite different: 1 alcohol, 2 phenols, 1 phenylpropanoid, 1 ketone, 23 terpenes were not detected at besser stage in the study of El Arem et al.[1,7]. It may be explained by the difference in the cultivars and/or the harvest locations.

For the seeds, only Saafi-Ben Salah et al.[30] has determined the volatile profile in 7 tunisan varieties. According to this study, 34 terpenes were not been previously identified (among which camphene, β-phellandrene, γ-terpinene, α-pinene and p-cymene were the most). These differences could be explained by the effect of the chosen cultivar and the harvest maturation stage.

As secondary metabolite data, Chaiera et al.[4] affirmed that the common variety Korkobbi produced a good amount of phenolic compounds at tamr stage. Previous studies showed that the phenolic compounds of date fruits decreased during the maturation stage and the khalal stage was the polyphenols-richest edible stage. That could be explained by the high tannins content of the date fruit at this stage of ripening. During the maturation, these polyphenols tend to decrease due to oxidation and enzymatic browning[31].

Our results confirmed the finding of Al-Ogaidi and Mutlak[32], who proved that the phenol content could reach 3000 mg/100 g at the earlier stages of maturity. The date fruit is a good source of polyphenols: at besser stage their levels are higher than those detected in wide range of fruits and vegetables such as raspberries, tomatoes, onion and apple[33].

As has already been demonstrated for other fruits and their corresponding seeds[34], total polyphenol content in the date seeds was higher than in the edible flesh. Their content is higher than even cocoa powder which is classified in fourth place in the database of polyphenols content of food sources[35].

Our results, 4210 mg GAE/100 g extract for Korkobbi seeds...
and 4431 mg GAE/100 g extract for Arechti seeds, are in great agreement with those of Al-Farsi et al. who reported 4430 mg GAE/100 g in Mabseeli seeds and 4293 mg GAE/100 g in Um-Sellah ones. Furthermore, they are much higher than those reported by Al Juhami et al. These differences may occur due to the different date varieties, geographical origin and harvesting time.

The seeds of Arechti and Korkobbi have also shown a great amounts of flavonoids and anthocyanins, higher than those detected in the flesh of the same cultivars.

Our levels of flavonoids are higher than those reported by Mistrello et al. for the seeds of three date varieties (1271–1932 mg caffeic acid equivalent/100 g). Flavonoids are naturally detected in different parts of plants such as fruits and seeds and they play an important role in the nutritional and organoleptic qualities of plants.

In addition, the seeds of our two varieties contained appreciable amounts of condensed tannins, with average values of 2013 and 2681 mg CE/100 g extract. Tannins are greatly involved in the bitterness and the astringency of the seeds, the protection against pathogen attacks and the astringency mechanisms.

Plants produce different types of secondary metabolites playing a vital and significant action in the neutralization or inhibition of the free radicals, related to many dangerous diseases. The interest in natural antioxidants is growing more and more in comparison to some synthetic antioxidants that may exhibit toxicity, have high manufacturing costs, and have lower efficiency.

In comparison with others fruits such as figs, prunes, and raisins, dates are good sources of antioxidants. With regard to by-products, seeds had the highest antioxidant activity. It has been shown that date pits have the potential to be used as a supplement for antioxidants in nutraceutical, pharmaceutical, and medicinal products.

The results obtained in this study demonstrate that the two common varieties and their seeds, on the basis of their nutrient composition, the important amount of volatiles and the variety of phytochemicals produced, can be effectively useful to protect the body from oxidative damage by free radicals scavenging activity and can be used as a source of safe and natural antioxidant compounds. For all these reasons, they could find applications both in the field of food industry and for the general use, likewise the most valued varieties.

In future, further studies should be conducted to establish the antioxidant mechanism of common date fruits and seeds extracts.

**Conflict of interest statement**

We declare that we have no conflict of interest.

**Acknowledgments**

This work was supported by Research Laboratory of Bioressources valorization (LR14ES06), Monastir, Tunisian Ministry of Higher Education and Scientific Research. The authors would like to thank all of the colleagues and students who contributed to this study.

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