Title: Antioxidant phenolic extracts obtained from secondary Tunisian date varieties (*Phoenix dactylifera* L.) by hydrothermal treatments.

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ABSTRACT

Three common non-commercial Tunisian date varieties were treated by two thermal systems, obtaining a liquid fraction which was characterized and its antioxidant capacity was determined. The concentration of total phenols in the three varieties (Smeti, Garen Gazel, & Eguwa) was increased by steam explosion treatment up to 5311, 4680, and 3832 mg/Kg of fresh dates, and their antioxidant activity up to 62.5, 46.5 and 43.1 mmol Trolox®/Kg of fresh date, respectively. Both thermal treatments increased the content of phenolic acids.

Additionally, a long scale study was carried out in a pilot plant with steam treatment at 140 and 160 ºC for 30 minutes. The liquid phase was extracted and fractionated chromatographically using adsorbent or ionic resins. The phenolic profiles was determined for each fraction, yielding fractions with interesting antioxidant activities up to EC_{50} values of 0.08 mg/L or values of TEAC of 0.67 mmol Trolox®/g of extract.

Keywords: date, antioxidant, phenolic extract, thermal treatment.

1 INTRODUCTION

Natural antioxidants are gaining an ever increasingly important role in the food industry with customer-drive pressure to replace the use of synthetic additives in food products to include natural ones, and importantly, to impact their well-documented protective effects against illnesses such as cancer and cardiovascular diseases (Harasym & Oledzki, 2014). A wide range of antioxidant extracts obtained from natural sources, including fruits, plants, or agro industrial wastes such as the semi-solid by-product from the olive oil production process, are been studied to establish their biological properties (Kahkonen et al., 1999, Fernández-Bolaños, Rodríguez, Rodríguez, Heredia, Guillén &. Jiménez, 2004). In some cases, the extraction of these components helps to revalorize agricultural wastes or even secondary cultivars that are at risk of disappearing. Palm dates are one promising food source of valuable compounds with antioxidant and antibacterial properties, for example polyphenols (Al-Farsi, Alasalvar, Morris, Baron & Shahidi, 2005, Biglari, Alkarkhi & Easa, 2008, El-Azim, El-Mesalamy, Yassin, & Khalil, 2015). The fruits of the date palm (*Phoenix dactylifera* L.) are commonly consumed worldwide, and are the most important commercial crop in the Arab World (El-Rayes, 2009) however, not all the varieties are been commercialized as some do not have sufficient commercial quality. Dates are one of the main crops in Tunisia, where there are many commercial varieties, such as Deglet Nour, Allig,

Kentichi, etc., but there are also many other non-commercial varieties that are progressively disappearing. Secondary cultivars are characterized by a low commercial quality and, although they are not commercially viable cultivars for human food consumption, they could be an important source of natural bioactive compounds for application in the food industry. Thus, there is a pressing need to study the properties of the non-commercial varieties, of which only limited data is available regarding their compositional characteristics (Mrabet, Rodríguez-Arcos, Guillén-Bejarano, Chaira, Ferchichi & Jiménez-Araujo, 2012, Mrabet et al., 2015). Furthermore, since the cultivation of dates represents a major source of income for the majority of the rural population and many non-commercial varieties have been developed in local areas as secondary crops, the valorization of these varieties to convert these unused varieties into value added products would help the local economy.

The antioxidant activity of the date palm is attributed to its phenolic composition, including ρ-coumaric, ferulic, and sinapic acids, flavonoids, and procyanidins (Hong, Tomas-Barberán, Kader, & Mitchel, 2006). In order to extract these components from the palm date, a liquid source is required in which the phenols have been solubilized, using aqueous or organic solvents, and applied temperature would enhance the extraction. In a previous work, a hydrothermal system was used to treat the non-commercial date varieties from Tunisia. The hydrothermal treatment successfully solubilized phenolic compounds in the liquid phase (although the liquid fractions were not further analyzed) and left a solid fraction rich in antioxidant fiber (Mrabet et al., 2015). In this study, two different treatments were applied to samples from secondary Tunisian date varieties, steam explosion (SET) in which a high temperature and pressure was applied, followed by an explosive decompression, and steam treatment (ST) in which lower temperature and pressure conditions were used without explosion. These treatments cause the solubilization of sugars and phenols in the liquid phase and have been widely studied for the treatment of olive oil wastes, with the ST method used

78 industrially by the pomace olive oil extractor (Fernández-Bolaños, Rodríguez, Lama &
79 Sánchez, 2011).

80 The aim of this study was to assess the effect of the two thermal pre-treatments on the
81 previously uncharacterized liquid fraction obtained from hydrothermally treated secondary
82 varieties of dates. This work complements the previous valorization of the solid extracts of
83 these secondary cultivars (Mrabet et al., 2015). The composition, including the contents of
84 total sugar, uronic acid, and degradation products, phenolic profiles, and antioxidant
85 capacities of the liquid fraction obtained by different treatments following fractionation for
86 evaluating the antioxidant activity of each fraction using adsorption and ionic
87 chromatographic systems were determined. Finally, the possible commercial applications of
88 the bioactive compounds extracted from the liquid phase of hydrothermally treated dates
89 from secondary varieties will be discussed.
90

91 ## 2 MATERIALS AND METHODS

92 ### 2.1 Materials

93 Three secondary palm date varieties (Garen Gazel, GG, Eguwa, EG, and Smeti, SM) at the
94 "Tamr stage" (full ripeness) that contain proved antioxidant components were studied
95 (Mrabet et al., 2015). They were picked at Gabès littoral oasis (southern Tunisia) during the
96 2011 harvest season (September-October). All samples were stored at -20°C until analysis
97 and treatment.
98

99 ### 2.2 Thermal treatments

100 *Steam explosion treatment (SET).* The dates were cut longitudinally to improve the access of
101 steam to the fruit. Date samples of 250 g were treated with saturated steam in a 2 L reactor

with a maximum operating pressure of 42 Kg/cm^2. The reactor was equipped with a quick-opening ball valve and an electronic device programmed for the accurate control of steam time and temperature for the final steam explosion. Two temperatures were used, 180 and 200 ºC for reactions of 5 minutes, based on previous studies (Mrabet et al., 2015). After the treatment, the samples were collected and vacuum filtered through filter paper using a Buchner funnel, and stored at -20ºC until analysis.

Steam treatment (ST). ST without explosion was carried out using a 100-L reactor, which can operate at temperatures between 50 and 190 °C by direct heating, and at a maximum pressure of 9 Kg/cm^2. The system allows the appropriate treatment of dates without explosion or high pressures and temperatures. The conditions used were 165 and 180 ºC in the first study and 140 and 160 ºC in the second for the fractionation. All the treatments were carried out for a 30 minute reaction time. The wet treated material was filtered by centrifugation at 4700 g (Comteifa, S.L., Barcelona, Spain) to separate the solids and liquids, and the samples were stored at -20ºC before analysis and fractionation.

2.3 Phenol extraction

The phenolic extracts were made from the date samples thermally treated using ethyl acetate as a solvent, and the control were obtained from the untreated date samples using ethanol.

Ethanol extraction of untreated dates. One gram of date flesh was extracted twice with 100 ml 80 % ethanol at room temperature. The liquid was collected and made up to 200 ml in a volumetric flask to measure the total phenols and soluble antiradical activity as a control.

Organic extraction of thermally treated date. After the thermal treatment, the liquid phase was extracted with ethyl acetate (refluxed at 77 ºC) for 5-6 h in a continuous extraction from the heavier liquid (water) to the lighter one (ethyl acetate). The organic phase was vacuum evaporated at 37 ºC to obtain the dry phenolic extracts.

2.4 Determination of sugars

The total neutral sugars and uronic acids in each liquid fraction obtained in the first study were assayed using the anthrone-sulphuric acid colorimetric assay at 520 nm (Dische, 1962) and the m-hydroxyphenyl method measuring the absorbance values at 620 nm (Blumenkrantz & Asboe-Hansen, 1973) in an iMark^{TM} microplate absorbance reader (Bio-Rad, Hercules, CA, USA).

2.5 Determination of total phenols

Total phenolic content was determined by the Folin-Ciocalteu spectrophotometric method and was expressed as grams of gallic acid equivalents (Singleton & Rossi, 1965).

2.6 Analysis of phenols by HPLC-DAD

Phenols were quantified using Hewlett-Packard 1100 liquid chromatography system with a C-18 column (Teknokroma Tracer Extrasil ODS-2, 250 mm x 4.6 mm, i.d. 5 μm) and diode array detector (DAD, the wavelengths used for quantification were 254, 280, and 340 nm) with Rheodyne injection valves (20 μL loop). The mobile phase were 0.01 % trichloroacetic acid in water and acetonitrile utilizing the following gradient over a total run time of 55 min: 95 % A initially, 75 % A in 30 min, 50 % A in 45 min, 0 % A in 47 min, 75 % A in 95 min, and 95 % A in 52 min until completion of the run. Quantification was carried out by integration of the peaks at different wavelengths in function of the compounds, with reference to calibrations made using external standards.

2.7 Chemicals

Hydroxymethylfurfural (HMF), furfural, vanillic acid, p-coumaric acid, protocatechuic acid, syringic acid, and trichloroacetic acid were obtained from Sigma-Aldrich (Deisenhofer, Germany). Tyrosol was obtained from Fluka (Buchs, Switzerland). HPLC-grade acetonitrile was purchased from Merck (Darmstadt, Germany) and ultrapure water was obtained using a Milli-Q water system (Millipore, Milford, MA, USA). The extraction solvents ethyl acetate and methanol were obtained from Romil Ltd. (Waterbeach, UK).

2.8 Fractionation of samples

The samples obtained by ST at 140 and 160 ºC for 30 minutes were fractionated to obtain phenolic extracts by one chromatographic column, using either adsorption or ionic resins. A volume of 150 mL of each liquid fraction was passed through each column, and four different fractions (F1 to F4) were collected. All fractions were analyzed to determine the total phenols during the chromatographic elution using a gradient of methanol: water (from 100% of water up to 100% of methanol) in the case of adsorption onto an Amberlite XAD-16 resin, or only water for the fractionation with ionic resins (IRA 4200Cl anionic resin).

2.9 Determination of the antiradical activity

Antiradical activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH). The antioxidant activity of each liquid phase obtained by SET and ST after fractionation was determined as the free radical-scavenging capacity using the DPPH method described in a previous study (Rodríguez et al., 2005). The radical-scavenging capacity of each antioxidant was expressed as EC_{50} (effective concentration, mg/mL), as calculated from a calibration curve using linear regression for each antioxidant.

Antiradical activity: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS). The antioxidant capacity was also determined for the fractionated phenolic extracts after ST at

lower temperatures by measuring the radical-scavenging capacity with the ABTS method. The ABTS assay was performed according to the method of Gonçalves, Falco, Moutinho-Pereira, Bacelar, Peixoto and Correia (2009) with some modifications as described in a previous work (Rubio-Senent, Rodríguez-Gutiérrez, Lama-Muñoz & Fernández-Bolaños, 2012). The results were expressed in terms of the Trolox equivalent antioxidant capacity (TEAC) in mmol Trolox®/g of extract.

2.10 Statistical analysis

Results were expressed as mean values ± standard deviations. STATGRAPHICS® plus software was used for statistical analysis. Comparisons amongst samples were made using one-way analysis of variance (ANOVA) and the LSD method. A p-value of 0.05 was considered significant.

3 RESULTS AND DISCUSSION

3.1 Hydrothermal treatments for liquid extract

Date samples from three secondary Tunisian date varieties were subjected to two different hydrothermal treatments, steam explosion (SET) and steam treatment (ST). In the former, the three different date varieties were each studied at 180 and 200 ºC for 5 min, the latter used lower temperatures (165 and 180 ºC) for treatment times of 30 min, without explosion, and required higher quantities of samples, hence it was necessary to mix the three varieties.

3.1.1 Phenolic composition of liquid fraction.

199 The chemical composition of the solid phase obtained from the date fruits of the three
200 varieties with and without thermal treatment has been previously studied (Mrabet et al., 2012,
201 Mrabet et al., 2015). In the present work, the phenolic profiles of the liquid fraction were
202 determined by HPLC using standards commonly present in other commercial dates (Al-Farsi
203 et al., 2005, El-Rayes, 2009). The total sugar and the uronic acid composition were also
204 determined to show the effect of the thermal treatment on phenol solubilization into the liquid
205 phase. The total sugar content and the concentration of the uronic acids as acid sugars are
206 shown in **Table 1**. There are notable differences between samples from the three varieties and
207 between the different thermal treatments, with the highest concentration of total sugars and
208 uronic acids obtained from the SM variety at 180 ºC SET. The sugar content diminished for
209 all three varieties with the severity of the SET, halving the concentration of sugars for the SM
210 and GG varieties. The behavior and the concentration of the acid sugars for the three varieties
211 assayed, measured by uronic acid, differed considerably with the severity of the thermal
212 treatment, with SM the most affected and EG the least affected, although all three showed
213 lower concentrations of uronic acids at the higher temperature of SET.
214 The total phenols and the main phenolic compounds present in the samples of SM, GG, and
215 EG date varieties treated by SET at 180 and 200 ºC for 5 min and the mix of the date samples
216 treated by ST at 165 and 180 ºC are also shown in **Table 1**. In **Figure 1**, the average
217 chromatographic profile of the liquid fraction obtained after the thermal treatment of dates is
218 shown. The main phenols identified (numbered) are tyrosol and the phenolic acids like gallic,
219 protocatechuic, vanillic, or p-coumaric acids, besides the sugar degradation products. The
220 presence of degradation products of sugars, like hydroxymethylfurfural (HMF) and furfural,
221 is representative of the severity of the thermal treatment applied. Higher amounts of HMF
222 were found with SET compared to ST, and especially at the higher temperature of 200 ºC,
223 with higher temperature having a greater effect than longer reaction time. However, this was
224 not the case for furfural, SET led to lower concentrations of furfural in the liquid phase than

ST. This is because furfural is a volatile compound that can easily volatilize in the expansion step of the SET while the volatilization of these compounds is lower in the ST in which no expansion and higher samples volumes are used. The total phenol contents of the SM, GG, and EG varieties increased with the severity of the SET, up to 10, 56 and 61%, respectively, as well as the concentration of all the phenolic compounds. Gallic acid was the only phenolic compound whose concentration decreased with SET at the increased temperature. The increase in phenol concentration in date samples after thermal application was previously reported (Allaith, Ahmed & Jafer, 2012) for a temperature of 100 ºC. The total soluble phenolic content of the untreated dates extracted by alcoholic solution was 160.3, 866.2, and 427.0 mg/Kg for GG, EG and SM varieties, respectively. These values were widely exceeded by all thermal treatments, meaning the hydrothermal treatments employed help to solubilize a higher quantity of phenols from dates than the organic extraction commonly used for the raw material characterization. No significant differences were found between the phenolic contents of the three varieties apart from the considerably higher concentration of tyrosol (1.7g/Kg) obtained after SET at 200 ºC for GG. Interestingly, our previous study of the solid phase after thermal treatments also found no differences between the phenol compositions of the solid phases of the three date varieties (Mrabet et al., 2015). Different to SET, the increased temperature of the ST decreased the total phenol content by 25%, and the concentration of all phenolic compounds decreased except p-coumaric acid, which was the only phenol to increase with more severe ST by some ten-fold. For ST, the use of 165 ºC allowed for a richer phenol liquid to be obtained than at the higher temperature. The date sample used for ST was a mix of the varieties, hence the results are not directly comparable with the results of each variety treated by SET, however, the trends show that SET was the more effective thermal treatment for phenol solubilization in the liquid fraction albeit a more technically complicated system to scale up to the industrial level.

3.1.2 Antiradical activity of liquid treated.

The antiradical activity of the three thermally treated date varieties (**Table 1**) showed no significant differences between the two temperatures used in SET. The SM variety showed a higher antioxidant activity than the other two. The values of antioxidant capacity for the three untreated fresh date varieties obtained after the ethanolic extraction were 50.4, 48.2 and 81.2 of mmol Trolox®/Kg for SM, GG, and EG varieties, respectively. Comparing these values with those obtained for the liquid fraction following thermal treatments, SET only enhanced the antioxidant activity of the SM variety, the antioxidant activity of GG was maintained, and for the EG variety, it diminished. Nevertheless, thermal treatment allows a liquid source to be obtained from which it is easier to extract the phenols and avoids the use of organic extraction.

The antioxidant activity values obtained for ST were lower than for SET ones, with no significant differences observed between 180 and 165 ºC despite the differences caused to the phenol concentrations at the higher temperature.

3.2 Pilot ST for fractionation study

The results of the preliminary study using the hydrothermal treatment of dates show that the best condition for phenol extraction was ST at 165 ºC. By fractionating and evaluating the antioxidant activity of each fraction, we studied the role of components or group of components in the total activity. The ST at 165 ºC produced a high total sugar yield and antiradical activity, yet low degradation products, and importantly, obtained a large quantity of phenolic compounds. Furthermore, its industrial implementation is technically and economically more viable than the higher temperatures and pressures of SET.

3.2.1 ST and chromatographic fractionation.

Samples of several secondary date varieties were mixed and treated by ST at the lower temperature of 140 or 160 ºC for 30 minutes. These gentler conditions were tested in order to compare a temperature close to the best conditions as previously determined. After each thermal treatment, the solid and liquid phases were separated and 150 mL of the liquid phase was chromatographically fractionated using adsorbent or ionic resins. The fractionation was made in order to study the contribution of each fraction to the antioxidant activity. The balance of total phenols extracted by each chromatographic system is shown in **Table 2**. The use of a thermal treatment of 140 ºC yielded double the concentration (up to 7 g/L) after the chromatographic extractions of total phenols from date that obtained from the higher temperature treatment. The adsorbent resin retained a significantly higher quantity of total phenols, close to double the amount retained by the ionic resin. The results also showed that the ionic resin eluted ten times less than the other adsorbent resin in the volume used for the elution. This may be because the volume used for elution is greater for the ionic elution. Despite the differences noted in the total phenol concentration in the initial liquid fraction with ST temperature, the quantity of phenols eluted were similar for both the temperatures, over 400 and 40 mg for the adsorption and the ionic resin, respectively.

The elution profiles for the two resins were also different (**Figure 2**). The use of an alcoholic gradient in the adsorption resin led to the production of a curve with a maximum close or slightly higher than 100 mg of total phenols, for the elution profile of the ionic resin, a rapid decrease in the total phenols extracted in each fraction was observed, and less than 2.5 mg of total phenols were recovered from the remaining fractions.

The total phenol content of the fractions obtained after the chromatographic separation is listed in **Table 3.** Four fractions were obtained using the adsorption resins after fractions with similar chromatographic profiles were combined, three in the case of the ionic resins. As previously mentioned, the total phenol content was higher in the case of the adsorption resin. The concentration of phenols diminished during each elution for the ionic resin yet increased

in the case of the adsorption resin for the 160 ºC sample. A maximum concentration was found for the adsorption resin in the first fraction after ST at 140 ºC but found in the second fraction after ST at 160 ºC (with high amounts also found in the third fraction for 160 ºC). There were also three fractions obtained (two from adsorption and one from ionic resins) with a percentage of phenols greater than 50% referred to dry matter.

In **Table 4**, the concentrations of all the identified phenolic compounds and degradation products, as well as their antioxidant activities are showed for each fraction. Results are indicated for the same volumes (initial volume) for each fraction in order to compare then directly. The HMF and furfural concentrations are at their highest in the first fractions for each resin and diminish in subsequent fractions. Likewise, the content of gallic acid is also highest in the first fractions and diminishes with subsequent elutions, different from the rest of the identified phenols that are not present in all the fractions. For example, protocatechuic acid is concentrated mainly in the fractions A140-2 and A160-2 for the adsorption resin and I140-2 - I160-2 and 3 for the ionic resin, whereas the content of tyrosol is significant in the case of the 160 ºC fractions and is present in the A160-2 and I160-2 fractions. The vanillic, syringic, and p-coumaric acids are present in the same fractions, the last of each elution.

3.2.2 Antiradical activity of the liquid phase and each fractioned extract.

The antiradical activity of each fraction of the liquid phase eluted from the two types of resins and the initial liquid phases obtained after the thermal treatment was determined by two measures, using the DPPH and ABTS methods, and the results were expressed as EC_{50} and TEAC. The EC_{50} and the TEAC values (**Table 4**) show in the case of the adsorption resin that, except for one fraction in each temperature, the fractions have significantly higher activities than the unfractionated liquid treated at the two temperatures (D fractions). For the adsorbent resin, the EC_{50} values diminish significantly in the case of the liquid phase from treatment at 140 ºC (A140) meaning the antioxidant activity increased in the fractions up to

328 A140-4 and was associated with a total phenolic content of 22 % referred to dry matter (see
329 **Table 3** for corresponding phenolic content values). For the liquid phase from dates treated at
330 160 ºC, the EC_{50} values show a similar result except for a maximum for the fraction A160-4,
331 which also had the maximum percentage of phenols (64.9% referred to dry matter). All the
332 ionic resin fractions have significantly higher activities than the unfractionated liquid treated,
333 and the EC_{50} values diminished up to the third fraction, I140-3 and I160-3 had lower phenolic
334 concentrations than those obtained using the adsorbent resin and a higher antioxidant activity.
335 The TEAC values showed a similar activity to the EC_{50}, with the antioxidant power of each
336 fraction increasing during the elution in both resins. Maximum TEAC values were obtained
337 for the ionic fractionation of the liquid phase from the 160 ºC treatment (I160-2 and I160-3
338 were richer in protocatechuic, vanillic, syringic, and p-coumaric acids). In both antioxidant
339 measures, the best results were obtained for the fraction I160-3, which had the highest TEAC
340 and lowest EC_{50}, obtained after the ionic resin fractionation of the liquid phase from thermal
341 treatment at 160 ºC.

342 The phenolic contents of the untreated date varieties (Smeti, Garen Gazel, and Eguwa) are
343 lower than that of some commercial date varieties, which have an average range of 2000-
344 3000 mg/Kg of fresh fruit (Singh, Guizani, Essa, Hakkim & Rahman, 2012, Ardekani,
345 Khanavi, Hajimahmoodi, Jahangiri & Hadjiakhoondi, 2010, Saafi, El Arem, Hammami &
346 Achour, 2010, Al-Farsi et al., 2005, Biglari et al., 2008) or maximum values in the range of
347 4880-4559 mg/Kg of fresh weight for Gur and Adja cultivars, respectively (Saleh, Tawfik &
348 Abu-Tarbouch, 2011, Al-Turki, Shahba & Stushnoff, 2010). However, both the SET and the
349 ST produced a liquid phase rich in valuable components, like phenols and sugars, with a
350 higher content of phenolic acids than other varieties analyzed without treatment (Al-Farsi et
351 al., 2005, El-Rayes, 2009). The concentration of total sugars was higher than 25 g/L in this
352 discontinuous system, and could increase further in a continuous system, making these date
353 varieties a natural source of sugars for different purposes, such as inclusion in animal feed or

for the application of bioprocesses for energy production or ethanol production besides others, although it would be important to previously remove the presence of toxic compounds, mainly phenols and sugar degradation products, prior to fermentation (Oliva, Ballesteros, Negro, Manzanares, Cabañas & Ballesteros, 2004). On the other hand, the recovery of phenols would not only reduce the toxicity of the liquid phase of thermally treated dates for subsequent fermentation processes, but would also allow the extraction of bioactive phenolic compounds with antioxidant properties as a value added product. BHT and TBHQ are synthetic antioxidants added to food to prevent rancidity in fats and oils, and widely used in both the human food and animal feed industries. A correlation between antioxidant capacity and phenolic content was not found, either SET or ST. The antioxidant assays of the extract obtained after the pilot ST using optimized conditions, showed that some fractions had DPPH antiradical activity similar to that of commercial antioxidants, like BHT (EC_{50} 0.283 mg/L) or TBHQ (EC_{50} 0.115mg/L) (Olszewska, 2011), which, in the case of the I160-3 fraction (EC_{50} 0.08mg/L), was even higher. For the ABTS radical scavenging test, the values obtained for thermally treated date were similar to BHT (TEAC of 0.55 mmol Trolox®/g) for fractions I160-2 and I160-3. The fractions with the highest antiradical activity were obtained using ionic resins and the phenol content did not influence the antioxidant results. In comparison with other natural extracts, the activity showed for the fractioned extracts of treated dates are higher than the results obtained for grape seed (Li, Wang, Li, Li & Wang, 2008) but lower than other thermally treated agroindustrial by-products such as olive oil waste solid, alperujo(Rubio-Senent et al., 2012). In comparison with other Tunisian date varieties (Khouet Kenta, Kentichi, Deglet Nour or Allig, with values of EC_{50} for the DPPH test of 0.53, 0.61, 0.69, and 1.4, respectively) (Saafi et al., 2009), the EC_{50} values obtained using the pilot thermal reactor with a mix of the studied secondary date varieties was lower in the unfractionated sample but similar or higher in some fractions.

4 CONCLUSION

The steam treatment of secondary varieties of Tunisian date fruits could be an interesting alternative for local date utilization to prevent these date varieties loss. The thermal treatment and fractionation of dates allows for the removal of the toxic components, to yield a functional solid extract and a final liquid phase that is enriched in sugars and antioxidant phenolic compounds that could be a valuable ingredient for the formulation of healthier foods. Further studies could be carried out to fully characterize the types of sugar, in terms of poly and oligosaccharides and their biological activities. Finally, the steam treatment conditions were studied for phenolic extraction from dates, lowering the reaction temperatures (and utilizing much lower pressures than SET). In these conditions the system can be scaled up easily for industry, making the steam treatment of secondary date varieties to yield bioactive compounds for use in the food industry a viable source of income for rural Tunisian communities.

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Figure captions

Figure 1. Typical chromatographic profile (**A**) and its enlargement (**B**) of the liquid fraction obtained from the thermally treated dates at 180-200 ºC and the main compounds detected: 1: Gallic acid, 2: Hydroxymethylfurfural, 3: Furfural, 4: Protocatechuic acid, 5: Tyrosol, 6: Vanillic acid, 7: Syringic acid, and 8: p-coumaric acid.

Figure 2. Total phenols (mg) in each fraction eluted from adsorbent (**a**: liquid from treatment at 140 ºC, **b**: liquid from treatment at 160 ºC) and ionic resins (**c**: liquid from treatment at 140 ºC, **d**: liquid from treatment at 160 ºC).

Table 1. Phenolic composition, uronic acid and total sugar contents, and concentration of degradation products in the liquid fractions of different date varieties thermally treated by SET and ST. Both thermal treatments were done by duplicate and the analytical analysis by triplicate. Values are mean ± SD. Different letters indicate significantly different result ($p < 0.05$). nd. Value not determined. SM: Smeti, GG: Garen Gazel, EG: Eguwa.

	Steam Explosion Treatment						**Steam Treatment**	
	SM		GG		EG		Mix	
	180 ºC	200 ºC	180 ºC	200 ºC	180 ºC	200 ºC	165 ºC	180 ºC
Initial weight (g)	250.8	250.4	254.9	254.2	250.4	250.4	3950	6250
Liquid fraction (L)	4.4	3.2	3.0	3.1	2.8	3.7	28.3	31.9
Total sugars (g/kg)	537.7 ± 24.5	254.7 ± 9.2	418.8 ± 13.8	202.9 ± 11.1	309.1 ±24.8	248.2 ± 21.4	330.7 ± 9.6	181.7 ± 1.2
Uronic acids (g/Kg)	13.3 ± 1.2	5.8 ± 0.2	10.1 ± 0.2	6.4 ± 0.4	10.8 ± 0.5	9.3 ± 0.3	4.9 ± 0.1	2.4 ± 0.1
Phenols					***mg/kg***			
Gallic acid	1526.8 ± 23.1	1217.6 ± 43.3	1119.4 ± 17.5	1107.6 ± 37.4	1425.2 ± 25.4	1350.3 ± 31.0	688.8 ± 9.5	401.0 ± 4.7
Protocatechuic acid	308.3 ± 18.6	770.6 ± 74.4	368.1 ± 19.8	517.1 ± 25.5	380.1 ± 20.1	845.1 ± 36.9	224.54 ± 12.2	120.4 ± 10.9
Tyrosol	641.6 ± 102.3	978.4 ± 97.3	742.4 ± 83.6	1737.6 ± 106.1	564.6 ± 91.0	1251.7 ± 88.7	3.4 ± 0.5	nd
Vanillic acid	91.6 ± 6.9	217.9 ± 6.0	84.4 ± 9.5	77.4 ± 11.6	68.0 ± 5.4	45.4 ± 8.8	27.6 ± 3.1	13.9 ± 0.5
Syringic acid	63.9 ± 0.9	77.2 ± 5.8	53.0 ± 1.2	89.5 ± 2.9	72.7 ± 2.3	94.7 ± 4.5	25.1 ± 0.5	22.7 ± 0.1
p-coumaric acid	nd	120.6 ± 13.6	nd	150.2 ± 14.0	nd	96.2 ± 7.7	5.2 ± 0.9	53.3 ± 2.1
Total phenols (mg/kg)*	4828.0 ± 349.8	5311.1 ± 279.6	2993.4 ± 355.1	4679.8 ± 595.3	2372.8 ± 170.2	3831.7 ± 143.1	1513.7 ± 34.4	1128.4 ± 22.0
Degradation products					***mg/kg***			
Hydroxymethylfurfural	9004.2 ± 759.3	12507.4 ± 359.1	9541.9 ± 573.4	13157.1 ± 664.0	9183.5 ± 665.4	15230.4 ± 447.1	4791.9 ± 50.51	3687.2 ± 92.0
Furfural	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.0	0.3 ± 0.0	0.1 ±0.0	0.2 ±0.0	0.3 ± 0.0	0.6 ± 0.0
Antiradical activity *(mmol Trolox®/Kg of fresh date)*	**62.5 ± 4.1 c**	**52.1 ± 1.9 bc**	**46.5 ± 3.2 b**	**42.8 ± 0.5 b**	**43.1 ± 5.2 b**	**44.5 ± 4.4 b**	**21.4 ± 3.2 a**	**19.7 ± 2.5 a**

*Total phenols were determined by Folin-Ciocalteu method.

Table 2. Balance of total phenols using two chromatographic systems for phenol extraction of the two liquid extracts thermally treated at 140 and 160 ºC. Values are mean ± SD (measures were made by triplicate).

Resin	Temperature (ºC) of treatment for 30 min	Total phenol (mg/mL)	mg of phenol			% of total phenol charged	% of total phenol discharged
			Total (150 mL)	Retained	Eluted		
Adsorption	140	6.8 ± 0.7	1020.1 ± 64.3	800.1 ± 55.8	445.4 ± 17.6	78.4	43.7
	160	3.2 ± 0.2	480.0 ± 22.2	443.3 ± 25.0	412.7 ± 32.7	92.4	86.0
Ionic	140	7.1 ± 0.6	1065.1 ± 79.0	375.5 ± 12.9	44.3 ± 6.7	4.2	35.3
	160	3.7 ± 0.3	555.8 ± 33.1	271.5 ± 19.0	47.7 ± 4.3	8.6	48.8

*

Table 3. Fractions obtained after the two thermal processes (140 and 160 ºC) by chromatographic separation using adsorbent or ionic resins and their total phenolic content. Values are mean ± SD.

Resin	Temperature (ºC) for 30 min	Name	Fractions	Eluent (%of metanol in water)	Volume (mL)	Total phenol (mg/mL)	%Total phenol (referred to dry matter)
Direct	140	D-140	all	-	5860	0.18 ± 0.01	0.04
	160	D160	all	-	8775	0.06 ± 0.00	0.02
Adsorption	140	A140-1	F4 to F6	10-30	150	2.15 ± 0.05	24.7
		A140-2	F7	50	50	1.51 ± 0.05	23.6
		A140-3	F8 and F9	50-70	100	0.78 ± 0.02	56.0
		A140-4	F10 and F11	70-100	100	0.11 ± 0.01	22.2
	160	A160-1	F3 to F5	10-30	150	1.06 ± 0.04	12.3
		A160-2	F6 and F7	50	100	2.55 ± 0.11	24.3
		A160-3	F8	50-70	50	1.96 ± 0.13	23.9
		A160-4	F9 to F11	70-100	150	0.54 ± 0.04	64.9
Ionic	140	I140-1	F2 to F5	0	200	0.17 ± 0.00	62.2
		I140-2	F6 to F9	0	200	0.02 ± 0.00	13.4
		I140-3	F10 to F14	0	450	0.01 ± 0.00	10.4
	160	I160-1	F2 to F5	0	200	0.17 ± 0.00	36.7
		I160-2	F6 to F9	0	200	0.03 ± 0.00	8.5
		I160-3	F10 to F14	0	450	0.01 ± 0.00	6.4

Table 4. Phenolic profile and degradation products (hydroxymethylfurfural (HMF) and furfural) concentration of the fractions obtained after the two thermal processes (140 and 160 ºC) by chromatographic separation. The antioxidant measures are expressed as EC_{50} and TEAC of each fraction. Values are mean ± SD. Different letters (lower case letters for EC_{50} and capital letters for TEAC) indicate significantly different result ($p < 0.05$). D: un-fractionated samples, A: fractions from adsorbent resins, I: fractions from ionic resins.

Fraction	Degradation products (mg/L)		Phenols (mg/L)						Antioxidant activity	
									DPPH	ABTS
	HMF	Furfural	Gallic acid	Protocatechuic acid	Tyrosol	Vanillic acid	Syringic acid	p-coumaric acid	EC_{50} mg/L	TEAC (mmol Trolox®/g of extract)
D-140	4984.4 ± 24.3	0.2 ± 0.0	157.6 ± 3.8	19.4 ± 0.1	traces	3.7 ± 0.1	3.2 ± 0.1	0.8 ± 0.0	3.46 ± 0.18a	0.02 ± 0.00A
D160	9674.4 ± 32.0	1.4 ± 0.1	171.5 ± 5.5	51.8 ± 1.7	127.4 ± 3.7	1.6 ± 0.0	3.4 ± 0.1	2.3 ± 0.1	3.47 ± 0.08a	0.01 ± 0.00A
A140-1	4123.1 ± 21.6	0.2 ± 0.0	97.9 ± 3.0	-	traces	-	-	-	3.01 ± 0.03a	0.18 ± 0.01D
A140-2	654.0 ± 15.4	traces	54.2 ± 1.9	15.6 ± 0.8	-	traces	traces	-	1.04 ± 0.01cd	0.23 ± 0.01D
A140-3	traces	traces	35.4 ± 0.9	-	-	3.2 ± 0.1	3.0 ± 0.1	-	0.28 ± 0.00e	0.28 ± 0.01E
A140-4	-	-	12.0 ± 0.7	-	-	-	-	0.7 ± 0.0	0.16 ± 0.01e	0.22 ± 0.01D
A160-1	7843.1 ± 31.7	0.90± 0.0	114.8 ± 3.1	-	traces	-	-	-	2.14 ± 0.04c	0.08 ± 0.00AB
A160-2	841.0 ± 17.1	0.2 ± 0.0	46.1 ± 1.5	40.8 ± 2.0	118.5 ± 4.1	-	-	-	2.25 ± 0.08c	0.12 ± 0.00C
A160-3	traces	0.1 ± 0.0	8.4 ± 0.2	9.6 ± 0.4	traces	1.4 ± 0.0	-	-	2.39 ± 0.03c	0.21 ± 0.01D
A160-4	-	traces	3.2 ± 0.1	-	-	traces	2.8 ± 0.1	1.9 ± 0.1	0.77 ± 0.01d	0.23 ± 0.01DE
I140-1	2872.3 ± 12.6	0.1 ± 0.0	87.9 ± 2.7	-	-	-	-	-	1.44 ± 0.05c	0.08 ± 0.00 B
I140-2	319.3 ± 8.7	traces	35.6 ± 1.0	7.5 ± 0.3	traces	0.9 ± 0.0	1.2 ± 0.0	-	0.14 ± 0.01e	0.22 ± 0.01D
I140-3	traces	traces	12.1 ± 0.3	-	traces	-	0.1 ± 0.0	0.2 ± 0.0	0.11 ± 0.00ef	0.27 ± 0.01E
I160-1	6242.3 ± 19.7	0.4 ± 0.0	88.4 ± 2.2	traces	5.3 ± 0.3	-	-	-	1.61 ± 0.05c	0.08 ± 0.00B
I160-2	traces	traces	26.8 ± 0.9	4.5 ± 0.3	69.4 ± 2.1	-	-	-	0.15 ± 0.00e	0.39 ± 0.01EF
I160-3	-	traces	6.4 ± 0.1	9.4 ± 0.4	traces	2.1 ± 0.1	3.1 ± 0.1	1.4 ± 0.0	0.08 ± 0.00f	0.67 ± 0.01G

Figure graphics

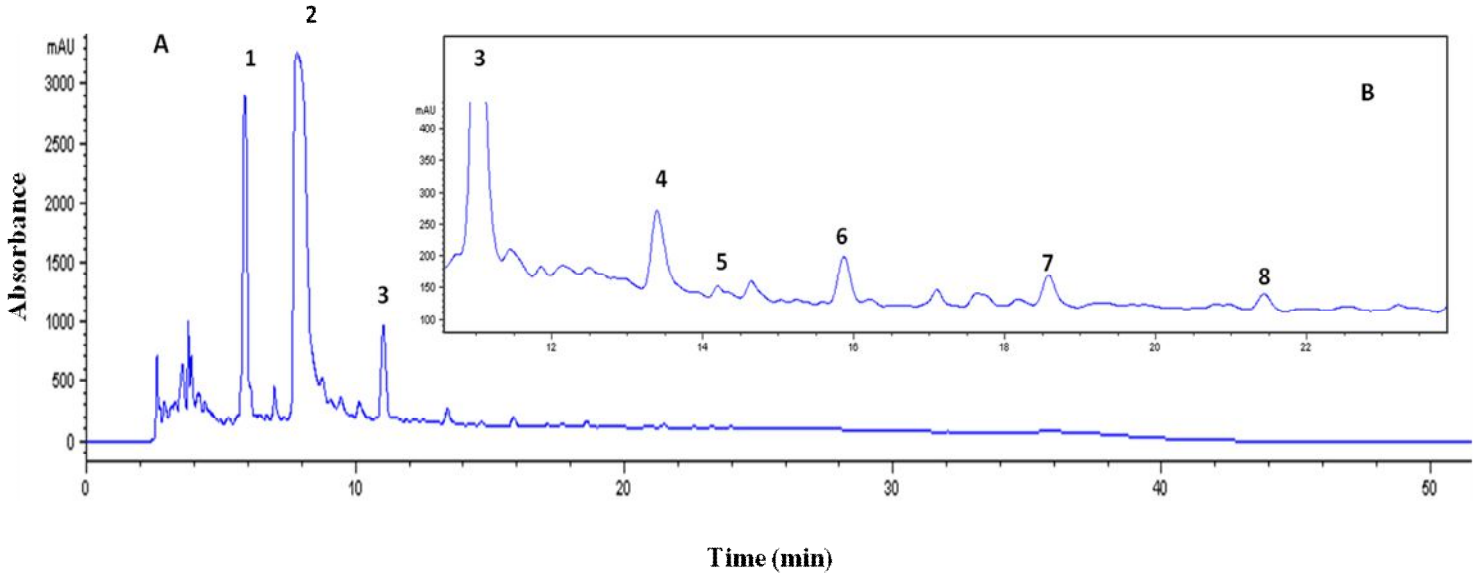

Figure 1.

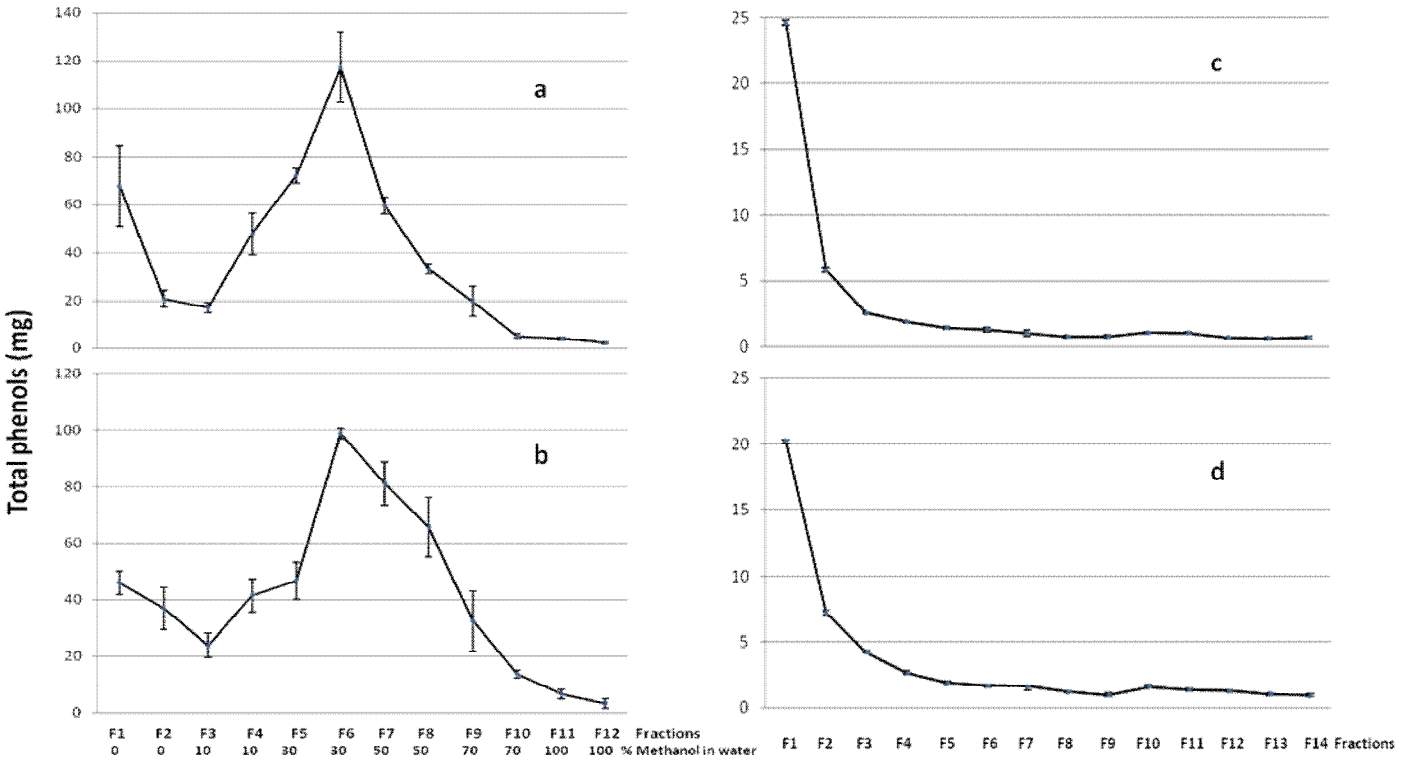
Total phenols (mg)
a
b
c
d
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 Fractions
0 0 10 10 30 30 50 50 70 70 100 100 % Methanol in water
F1 F2 F3 F4 F5 F6 F7 F8 F9 F10 F11 F12 F13 F14 Fractions

Figure 2.