



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 determined. The concentration of total phenols in the three varieties (Smeti, Garen Gazel, and 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 °C and 160 °C for 30 min. The liquid phase was extracted and fractionated chromatographically using adsorbent or ionic resins. The phenolic profiles were determined for each fraction, yielding fractions with interesting antioxidant activities with EC₅₀ values of up to 0.08 mg/L or values of TEAC of 0.67 mmol Trolox[®]/g of extract.

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1. Introduction

Natural antioxidants are gaining an ever increasingly important role in the food industry, with customer-driven pressure to replace the use of synthetic additives in food products with natural ones, and importantly, to implement 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, have been studied to establish their biological properties (Kahkonen et al., 1999; Fernández-Bolaños et al., 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 (Abed El-Azim, El-Mesalamy, Yassin, & Khalil, 2015; Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005; Biglari, Alkarkhi, & Easa, 2008). 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 have been

commercialized as some do not have sufficient commercial qualities. 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 et al., 2012, 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 also help the local economy.

The antioxidant activity of the date palm is attributed to its phenolic composition, including p-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 either 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

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

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

2. Materials and methods

2.1. Materials

Three secondary palm date varieties (Garen Gazel, GG, Eguwa, EG, and Smeti, SM) at the “Tamr stage” (full ripeness) that contain proved antioxidant components were studied (Mrabet et al., 2015). They were picked at Gabès littoral oasis (southern Tunisia) during the 2011 harvest season (September–October). All samples were stored at $-20\text{ }^{\circ}\text{C}$ until analysis and treatment.

2.2. Thermal treatments

2.2.1. Steam explosion treatment (SET)

The dates were cut longitudinally to improve the access of 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, $180\text{ }^{\circ}\text{C}$ and $200\text{ }^{\circ}\text{C}$, for reactions of 5 min, 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 $-20\text{ }^{\circ}\text{C}$ until analysis.

2.2.2. Steam treatment (ST)

ST without explosion was carried out using a 100 L reactor, which can operate at temperatures between $50\text{ }^{\circ}\text{C}$ and $190\text{ }^{\circ}\text{C}$ 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 $165\text{ }^{\circ}\text{C}$ and $180\text{ }^{\circ}\text{C}$ in the first study, and $140\text{ }^{\circ}\text{C}$ and $160\text{ }^{\circ}\text{C}$ in the second for the fractionation. All the treatments were carried out for a 30 min reaction time. The wet treated material was filtered by centrifugation at 4700g (Comteifa, S.L., Barcelona, Spain) to

separate the solids and liquids, and the samples were stored at $-20\text{ }^{\circ}\text{C}$ 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.

2.3.1. 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.

2.3.2. Organic extraction of thermally treated date

After the thermal treatment, the liquid phase was extracted with ethyl acetate (refluxed at $77\text{ }^{\circ}\text{C}$) 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 $37\text{ }^{\circ}\text{C}$ 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™ 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 \times 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 was 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).

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