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Phenolic extracts obtained from thermally treated secondary varieties of dates: Antimicrobial and antioxidant properties

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ABSTRACT

Palm dates are an important source of natural bioactive compounds and their application in the food industry could valorize secondary cultivars at risk of disappearing. Secondary palm date varieties were thermally treated by direct steam at two temperatures (140 and 160 °C) during 30 min. The liquid was extracted by organic solvent, obtaining two extracts, PE140 and PE160, with high concentrations of sugar degradation products, mainly hydroxymethylfurfural and phenolic compounds like tyrosol or gallic and protocatechuic acids. The use of the higher temperature produced 22 g of extract/Kg of fresh dates with antioxidant activities in the aqueous matrix (EC_{50} value of 2.72 mg/L for DPPH and TEAC value of 0.05 mmol Trolox®/g of extract) and remarkable protection against lipid oxidation in oils, increasing the stability of sunflower oil by the Rancimat method by four-fold. Promising values of inhibition against plant pathogens were found with values of MIC of 3.13 mg/mL. Therefore, thermal treatment of dates was effective for obtaining bioactive phenolic extracts suitable for food formulation.

1. Introduction

In the present day, both consumers and the food industry are giving considerable importance to healthy products obtained from natural sources. Consumers aim to diminish the use of synthetic additives or ingredients whose impacts on the health are not yet completely clear, whereas the trend in the food industry is to use natural components to obtain functional formulations with additional biological benefits besides the nutritional values. The current food industry trend is to use natural bioactive extracts to prevent the oxidation or the degradation of food, principally fat-based foods. In this sense, natural compounds are being used as antioxidants to control the development of oxidative rancidity in refined vegetable oils (Aluyor & Ori-Jesu, 2008; Carelli, Franco, & Crapiste, 2005) or fish oil (O'Sullivan, Mayr, Shaw, Murphy, & Kerry, 2005).

Natural, economic sources in which bioactive compounds are present in significant concentrations and can easily be extracted are required, such as food industry by-products (Putnik, Bursać Kovačević & Dragović-Uzelac, 2016). The utilization of waste or byproducts from the food industry can help to valorize them, not only with the extraction of bioactive compounds but also by avoiding environmental problems. Other kinds of natural sources include secondary varieties of fruits whose commercialization is diminishing because of other more commercially viable varieties. In some cases, these varieties are an important source of economic support for local regions, making their valorization an important issue to assist the social and economical development of certain areas. This is the case for secondary cultivars of palm dates from Tunisia (*Phoenix dactylifera* L.) that are at risk of disappearing. Previous studies about the utilization of these date varieties for sugar, fiber, and phenolic extraction have been carried out (Mrabet et al., 2015, 2016), which compared the direct use or the use after the application of a thermal pretreatment in food formulation. After thermal treatment, a liquid and solid phase are obtained: the solid phase is rich in fibers with antioxidant properties, and the liquid phase can be used as a source of phenols, obtaining an extract and a fraction with important antioxidant activities (Mrabet et al., 2016). The phenolic extracts of thermally treated dates were rich in tyrosol and phenolic acids like ferulic acid, sinapic acids, protocatechuic acid, and *ρ*-coumaric acid, beside degradation products of sugars

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such as hydroxymethylfurfural (HMF) or furfural, all of them with antioxidant activities (Mrabet et al., 2016). The hydrothermal treatment of dates causes the significant solubilization of bioactive and valuable compounds, leaving a final solid rich in cellulose and other degradable cell wall material. Similar thermal treatments have been successfully scaled up in the food industry, concretely in the olive oil industry, in which thermal treatment is starting to be used for the production of a liquid source of phenols from the final solid waste byproduct that are suitable for inclusion as bioactive ingredients (Fernández-Bolaños, Rodríguez, Lama, & Sánchez, 2011). The results obtained in previous work characterizing thermally treated secondary date varieties (Mrabet et al., 2016) showed an interesting phenolic fraction with antioxidant properties for both the more severe (180 and 200 °C for 5 min) and less severe treatments (165 and 180 °C for 30 min) at laboratory scale (reactor with 2 L capacity). These results lead to tests at the pilot scale (100 L reactor capacity) for two optimized treatment temperatures (140 and 160 °C), yielding two liquid extracts for each. Several fractions were produced and some antioxidant properties were determined *in vitro* (Mrabet et al., 2016).

The aim of this study is to increase our knowledge about the antioxidant properties on the lipophilic matrix using vegetable and fish oils and to study the antibacterial activity of the organic extracts obtained from hydrothermally treated secondary varieties of dates at the pilot scale using lower temperatures (140 and 160 °C) that can be easily scaled up for use in the food industry.

2. Materials and methods

2.1. Materials

A mix of three secondary palm date varieties (Garen Gazel, GG, Eguwa, EG, and Smeti, SM) at the "Tamr stage" (full ripeness) were picked at Gabès littoral oasis (southern Tunisia). The mix was made in order to diminish the variation between samples by cultivar and location (Al-Turki, Shahba, & Stushnoff, 2010). The antioxidant components of these varieties have been previously studied (Mrabet et al., 2016). All samples were stored at −20 °C until analysis and treatment.

2.2. Chemicals

The lipid matrix was obtained from commercial vegetable oils (olive oil, corn oil, sunflower oil, rapeseed oil, and palm oil) and fish oil (from menhaden) from Sigma-Aldrich (Deisenhofer, Germany). Hydroxymethylfurfural (HMF), furfural, vanillic acid, ρ-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 ethyl acetate was obtained from Romil Ltd. (Waterbeach, UK).

2.3. Thermal treatment

The steam treatment was performed using a prototype designed in the Instituto de la Grasa (Seville, Spain). The reactor has a stainless steel reservoir (100 L capacity) that can operate at temperatures between 50 and 190 °C and at a maximum pressure of 9 kg/cm^2. The conditions used were either 140 or 160 °C for 30 min, for a sample of 4 Kg of mix of fresh dates. The wet treated material was filtered by centrifugation at 4700*g* (Comteifa, SL, Barcelona, Spain) to separate the solids and liquids, and the samples were stored at −20 °C until analysis and extraction.

2.4. Phenol extraction

After thermal treatment, the liquid phases were extracted with ethyl acetate (refluxed at 77 °C) for 5–6 h in a continuous extraction from the water to the organic solvent. The organic phase was vacuum evaporated at 37 °C to obtain the two dry phenolic extracts, the extract obtained from the treatment at 140 °C (PE140) and that obtained at 160 °C (PE160), and stored at −20 °C until analysis.

2.5. Determination of total phenols

The total phenolic content of the two extracts was measured according to the Folin–Ciocalteu method and expressed as grams of gallic acid equivalents per kilogram of fresh dates (Singleton & Rossi, 1965) in duplicate.

2.6. Analysis of phenols and HMF by HPLC-DAD

The individual phenols and the HMF of each extract, PE140 and PE160, were quantified by HPLC (Hewlett-Packard 1100 liquid chromatography system) with a C-18 column (Teknokroma, Mediterranea Sea 18, 250 mm × 4.6 mm, i.d. 5 μm) and diode array detector (DAD, the wavelengths used for quantification were 280 and 340 nm) with Rheodyne injection valves of 20 μL. The mobile phases were 0.01% trichloroacetic acid in water, and acetonitrile. The gradient used for a total run time of 55 min was: 95% A initially, 75% A for 30 min, 50% A for 45 min, 0% A for 47 min, 75% A for 50 min, and 95% A for 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, in duplicate.

2.7. Determination of the antiradical activity

2.7.1. Antiradical activity: 2,2-diphenyl-1-picrylhydrazyl (DPPH)

The antioxidant activity of each phenolic extract was determined by the DPPH method as described in a previous study (Rodríguez et al., 2005). The method is based on the measurement of the free-radical scavenging capacity of the antioxidant against the stable radical DPPH. A microplate reader (Bio-Rad 550 model, Hercules, CA, US) was used for the absorbance measurements. The activity of each extract was expressed as an EC_{50}, the amount of antioxidant necessary to decrease the initial absorbance by 50% (effective concentration, mg/mL). The EC_{50} was calculated from a calibration curve by linear regression for each antioxidant solution.

2.7.2. Antiradical activity: 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS)

The radical-scavenging capacity was determined for both phenolic extracts with the ABTS method. The ABTS assay was performed according to the method of Gonçalves, Falco, Moutinho-Pereira, Bacelar, Peixoto & 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.

2.8. Evaluation of lipid oxidative stability by the Rancimat method

Lipid oxidative stability was evaluated by an accelerated automated test using the Rancimat equipment (Model 679, Metrohm Co. Basel, Switzerland). The lipid matrix was obtained from the commercial vegetable oils by purification through excess of alumina (1 g of alumina/2 mL of oil) to completely remove the antioxidants present naturally and the fish oil was used directly.