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Title: 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.

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

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 (Carelli, Franco & Crapiste, 2005; Aluyor & Ori-Jesu, 2008) 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; Mrabet et al., 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 p -coumaric acid, beside degradation products of sugars 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) at laboratory scale (reactor with two liter 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 of 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, *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 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/cm². The conditions used were either 140 or 160 °C for 30 minutes, 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, Mediterranean Sea 18, 250 mm x 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 μ L. 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

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 $\cdot$. 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 EC₅₀, the amount of antioxidant necessary to decrease the initial absorbance by 50 % (effective concentration, mg/mL). The EC₅₀ was calculated from a calibration curve by linear regression for each antioxidant solution.

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. The purified oils, free of antioxidants, were stored at -20 °C until analysis. Aliquots of 2.5 g of each oil were spiked with 100, 200, or 500 ppm of PE140 and PE160 (or 0 as a control) and then subjected to oxidation in the Rancimat apparatus. The temperature and air flow were adjusted for each oil in the range of 60–100 °C and 5–10 mL/min of air. The induction times were printed automatically by apparatus software with an accuracy of 0.005. Results were expressed as induction time (IT) in hours and the percentage in which this time was improved by the extracts. All determinations were carried out in triplicate.

2.9 Antimicrobial assays

Bacterial strains. The extracts were tested against eight bacterial strains obtained from the Spanish Type Culture Collection (CECT), University of Valencia. The reference strains were: *Xanthomonas campestris* (CECT 97), *Erwinia amylovora* (CECT 222), *Erwinia toletana* (CECT 5263), *Clavibacter michiganensis* subsp. *michiganensis* (CECT 790), *Clavibacter michiganensis* subsp. *insidiosus* (CECT 5042), *Rhizobium radiobacter* (CECT 4119), *Pseudomonas savastanoi* (CECT 5019) and *Pseudomonas syringae* (CECT 4429).

Antimicrobial activity assay. Antimicrobial activity was determined by the minimum inhibitory concentration test (MIC), defined as the lowest concentration of the extract showing no visible microbial growth after incubation time, following the growth dilution

method procedure described by Bassole, Ouattara, Nebie, Ouattara, Kabore & Traore (2003) and Hammer et al. (2008). Briefly, date extracts, which were soluble in water, were filtered through a sterile 0.2 μm filter. Twofold serial dilutions of the date extracts in Mueller Hinton broth were performed in 96-well round-bottomed microtiter plates ranging from 100 to 0.2 g/l of extract. Bacterial strains were cultured overnight in corresponding media. A solution from each strain in saline was adjusted to a final concentration of 10^6 colony forming units (CFU)/ml in each well. Wells containing 200 μl dilutions were inoculated with the corresponding bacterial suspension and aerobically incubated at 30 °C. The concentration of each inoculum was confirmed by a count of viable colonies on corresponding agar. Positive growth controls without date extract and negative controls without inoculum were included in each test. All the experiments were conducted in triplicate.

2.10 Statistical analysis

Results were expressed as mean values $\pm$ standard deviations. STATGRAPHICS® plus software was used for statistical analysis. Comparisons amongst samples were made using one-way analysis of variance (ANOVA) and the Fisher's least significant difference (LSD) method. A *p*-value of 0.05 was considered significant. Correlations between antioxidant capacity in oil by the Rancimat method and the polyunsaturated fatty acid: saturated fatty acid ratio (PUFA/SFA ratio) or the polyunsaturated fatty acid plus monounsaturated fatty acid: saturated fatty acid ratio (PUFA + MUFA/SFA ratio) were determined using Pearson's correlation coefficient test.

3 RESULTS AND DISCUSSION

3.1 Characterization of phenolic extracts

A mix of the three varieties of secondary Tunisian dates was subjected to hydrothermal treatment with steam at two temperatures (140 and 160 °C) for 30 minutes in the pilot plant reactor. The phenolic characterization of the extracts PE140 and PE160 is shown in **Table 1**. Treatment at the higher temperature led to a phenolic extract with five times the quantity but with a significantly lower content of total phenols. The difference in the volume of the liquid phase produced by each treatment is due to the condensation formation during the reaction's discontinuous process, with more condensation formed at the higher temperature. The composition of individual phenols in the extracts is similar to the previous characterization made of the thermally treated liquid phase (Mrabet et al., 2016) and to the phenols commonly present in other commercial date varieties (Al-Farsi, Alasalvar, Morris, Baron, & Shahidi, 2005; El-Rayes, 2009). Comparing the two treatments, there are significant differences in the quantities of individual phenols obtained from the two extracts (PE140 and PE160), mainly in the protocatechuic acid and tyrosol, and also in the vanillic and *p*-coumaric acids. Although the quantity of extract increased with the severity of the thermal treatment, the percentage of phenols decreased slightly. This could mean that the reaction solubilized a higher portion of the cell wall material, mainly hemicelluloses, which can be linked with simple phenols, thus explaining the reduction of these compounds. In fact, previous studies have reported the presence of antioxidant fiber in the solid fraction of thermally treated dates (Mrabet et al., 2015). This finding suggests the thermal reaction can enhance the bioactive compounds like oligosaccharides linked to the solubilized cell material. This fact has been reported by other authors who found neutral oligosaccharides, in addition to simple phenols like hydroxytyrosol or tyrosol with antioxidant capacities in olive oil by-products (Rubio-Senent, Lama-Muñoz, Rodríguez-Gutiérrez & Fernández-Bolaños, 2013). The use of thermal treatment has also been studied in asparagus, in which fiber with antioxidant capacity was

determined in the solid fraction rich in saponins and flavonoids (Fuentes-Alventosa et al., 2012). In addition, it has been reported that an ethyl acetate extraction process after hydrothermal treatment favors the formation of a polymeric phenolic fraction that has the ability to absorb simple phenols in other agro-industrial sources, like olive oil by-products (Rubio-Senent et al., 2012). The severity of the thermal treatment enhances the formation of this polymer that could absorb the simple phenols maintaining their antioxidant properties.

The most important difference between the two phenolic extracts is based on the production of a degradation product: the content of hydroxymethylfurfural (HMF) is high for the two temperatures used, but much higher for the 160 °C treatment (82.5 % compared to 59.7 % for treatment at 140 °C). The use of the higher temperature increased the content of HMF and furfural, although the majority of furfural was lost because of its volatility. These aromatic aldehydes are present naturally in food preparations like coffee, honey, dried fruits, fruit juices, and fruit flavoring agents (Xu, Li & Lü, 2007). HMF and furfural are also intermediate degradation products of hexoses and pentoses, respectively. HMF is a furanic compound that forms as an intermediate in the Maillard Reaction and from direct dehydration of sugars under acidic conditions (caramelisation) during the heat treatment of foods (Capuano & Fogliano, 2011). Thus, it is not surprising that the thermal treatments of secondary date varieties produce a high concentration of sugar degradation products, like HMF, which are concentrated in the phenolic extracts.

3.2 Antioxidant activity

Free radical methods. The *in vitro* antioxidant activities of the hydrophilic matrix were assayed by two different free radical methods, DPPH and ABTS (**Table 1**). The values of EC_{50} are slightly lower than the values (Mrabet et al., 2016) found previously in the liquid phase (the EC_{50} values were 3.46 and 3.47 mg/L, and the TEAC values were 0.02 and 0.01

mmol Trolox[®]/g for the liquid phase treated at 140 °C and 160 °C, respectively), and the antioxidant activities are higher because the phenols are partially purified during the extraction with organic solvent, removing some components that could have a negative antioxidant effect.

The antioxidant values of the two extracts cannot be explained by the main component, the HMF, whose antioxidant capacity is lower than the extracts. The antioxidant properties recently associated to HMF *in vitro* by ABTS and DPPH assays, showed an activity of 25 and 100 mg/L, respectively (Zhao et al., 2013). These values are higher than the values of EC₅₀ show in **Table 1** for PE140 and PE160, in which the presence of phenolic compounds must enhance the total antioxidant effects of the date extracts.

Rancimat method. Oils with different polyunsaturated fatty acid: saturated fatty acid ratios (PUFA/SFA ratio, P/S index) or polyunsaturated fatty acid plus monounsaturated fatty acid: saturated fatty acid ratios (PUFA + MUFA/SFA ratio, P+M/S index) were chosen. The balanced intake of dietary PUFA and SFA is thought to be very important in regulating factors like serum cholesterol (Lee et al., 1989); however, excessive intake of PUFA has undesirable effects such as oxidative stress (Park et al., 1999) because of high susceptibility to lipid peroxidation (Lee et al. 1989). Thus the use of natural antioxidants could help to diminish the lipid peroxidation of healthier oils.

The effect of the addition of the phenolic extracts to the oxidative stability of different oils was measured by the Rancimat method (**Table 2**). Five vegetable oils and one fish oil were tested with three concentrations of PE140 and PE160. Different conditions were used for the corn and the fish oils (80 °C and 60 °C, respectively) to increase the induction time to attempt to detect an effect following addition of the extracts. No effect was found for extract PE140 using corn, sunflower and rapeseed oils, and a positive effect in palm and fish oil was only

observed when the concentration used was 500 mg/L. The effect of the addition of both extracts to olive oil was positive and dose dependent, with the induction time increasing by up to 17% for the highest concentration. Better results for the accelerated oxidative conditions were obtained for the PE160 extract. In addition to a positive effect for olive oil, the extract showed an important increase in the induction time for all the oils tested, particularly for sunflower oil, for which the time was increased by four-fold for all the concentrations tested. To determine the influence of the main component of both extracts (HMF) in the induction time, similar concentrations of HMF present in each extract were tested in sunflower oil by the Rancimat method. All the results showed negative values close to zero or a zero percentage increase of induction time (data not shown). Because of the lack of effect of HMF, the presence of phenolic compounds must explain the remarkable effect of PE160 on sunflower oil protection. This result reveals extract PE160 as a potential agent to prevent oxidative damage in the studied oils, with a particularly striking effect in sunflower oil. Further studies will be required to determine the optimum concentration of PE160 to prevent oxidation in oils and to determine the effect during the storage of oils for commercial purposes. The negative influence of the HMF intake in food products has been widely reported (Capuano & Fogliano, 2011). This degradation product is present in carbohydrate-rich products in which the heat is applied during processing, and in ingredients used in the formulation such as caramel solutions or honey. The daily dietary intake of HMF has been estimated in a range of 2.1-23 mg for different scenarios (Rufian-Henares & De la Cueva, 2008). The remarkable protection of sunflower oil implies the use of 100 ppm of the date extract, meaning a maximum daily intake of 4 mg of HMF, for 50 g of oil/day.

The protection in lipid matrix depends not only in the antioxidant capacity of the extracts but also on the solubilization of the more active compounds in the oil. Many studies have been carried out in order to increase the solubility of phenols in oil making longer the lipophilic

structure linked to the phenol (Fernández-Bolaños, López, Fernández-Bolaños & Rodríguez-Gutiérrez, 2008). Increasing the number of C atoms in the alkyl chains of the natural antioxidant not only changes their hydrophilic–lipophilic balance but also affects their relative solubilities in the oil. For example hydroxytyrosol acetate shows a higher solubility and antioxidant activity in olive oil emulsions than the hydroxytyrosol (Lisete-Torres, Losada-Barreiro, Albuquerque, Sánchez-Paz, Paiva-Martins & Bravo-Díaz, 2012). The organic extraction with ethyl acetate by heating in acid conditions can produce the esterification of simple phenols, increasing their lipophilicity, from the liquid obtained after the thermal treatment of the dates. In addition, the severity of the thermal reaction can enhance the formation of the Maillard reaction products (MRP) with antioxidant capacities by a non-enzymatic reaction between reducing sugars and amino acids, peptides or proteins (Manzocco, Calligaris, Mastrocola, Nicoli & Lericci, 2001).

The relationship between the antioxidant capacity of the date extracts and the fatty acid ratios is shown in **Table 3**. The P+M/S and P/S ratios, and the average fatty acid composition have been obtained bibliographically (Kostik, Memeti & Bauer, 2013; Rahman & Salimon, 2006). No significant correlation was found using the P+M/S ratio. However, significant coefficients were obtained for the comparison of P/S ratio with the average and a maximum value of percentage increase (%Δ) for both phenolic extracts (140 and 160 °C). The coefficient $R_{P/S \text{ ratio}}$ shows a direct relationship between the antioxidant capacity of the extract obtained from the treatment at 160 °C and the degree of polyunsaturation of the analyzed oils. The relationship was inverse in the case of the extract obtained at 140 °C. Thus, the extract obtained at 160 °C seems to be the most appropriate to prevent lipid oxidation in polyunsaturated oils, with the activity shown in sunflower oil particularly of note, increasing the induction time by four-fold.

3.3 Antimicrobial assays

Minimum inhibitory concentrations (MICs) of date extracts PE140 and PE160 against eight bacterial strains are shown in **Table 4**. In all cases, the MIC values were lower for the extract obtained at 160 °C than for the extract obtained at the lower temperature, with the exception of the bacteria *Erwinia toletana*, for which the antimicrobial activity was the same for the two extracts. The highest antimicrobial efficacy was detected against *E. amylovora* and *C. michiganensis* subsp. *michiganensis* with a MIC value of 3.13 mg/mL for the extract obtained at 160 °C; on the contrary, PE160 was least effective against *E. toletana*, with a MIC value of 25 mg/mL. The extract obtained at 140 °C showed the highest efficacy against the strains *E. amylovora* and *C. michiganensis* subsp. *michiganensis*, with MIC values of 6.25 mg/mL, and was least effective against *X. campestris*, *C. michiganensis* subsp. *insidiosus* and *E. toletana*. The phytopathogenic bacteria used in this work belong to the category of gram-negative bacteria (except for the genus *Clavibacter*, which belongs to the gram-positive group), which are often more susceptible to antimicrobials. *C. michiganensis* subsp. *michiganensis* showed the lowest MIC values for both extracts, but not so with *C. michiganensis* subsp. *insidiosus*, which had similar MICs to the other gram-negative bacteria tested. In this paper, we did not detect differences in the effectiveness of the extracts related with the gram-category of the phytopathogens analyzed.

Our data indicate a slight antimicrobial value of the extracts analyzed, with a greater antimicrobial effect obtained for the extract from a thermal treatment of 160 °C. This could be due to the presence of hydroxymethylfurfural and other phenol-like compounds obtained after thermal treatment of foods exhibiting antimicrobial activity (Rufian-Henares & de la Cueva, 2009). Such compounds are more easily extractable under higher temperature conditions.

Despite the low percentage of total phenols present in each extract, the MIC values show the antibacterial potential of the extracts, particularly for PE160. In the case of the inhibition of *Clavibacter michiganensis* subsp. *michiganensis*, PE160 showed a similar effect to medical plant extracts used in Morocco as antimicrobial agents (Talibi et al., 2011). Thus, further studies are necessary to fractionate, analyze, and characterize the more antimicrobially active components of PE160.

4 CONCLUSION

The composition of the mixed date extracts show a high concentration of HMF as a main component, followed by phenolic compounds commonly found in dates. Thermal treatment enhances sugar degradation products, and the severity of the treatment improves the extract's antioxidant and antimicrobial properties. Therefore, the treatment is suitable for the production of oligosaccharides as well as phenols, esterified phenol, and MRPs. Both extracts showed similar antioxidant properties in the aqueous matrix, but the extract obtained after 160 °C treatment gave higher protection against peroxidation in oil, particularly for sunflower oil, and had a higher antimicrobial activity against plant pathogens. The lipid oxidation protection obtained was directly related to the polyunsaturated fatty acid of the oils.

Thus, the use of secondary varieties of dates after thermal treatment (160 °C, 30 min) leads to the obtention of about 22 g of phenolic extract/kg of fresh date with significant antioxidant and antimicrobial activities. Further studies are required to determine the active components that impart the antimicrobial properties.

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REFERENCES

- Al-Farsi, M., Alasalvar, C., Morris, A., Baron, M., & Shahidi, F. (2005). Comparison of antioxidant activity, anthocyanins, carotenoids, and phenolics of three native fresh and sun-dried date (*Phoenix dactylifera* L.) varieties grown in Oman. *Journal of Agricultural and Food Chemistry*, 53, 7592–7599.
- Al-Turki, S.M., Shahba, A., & Stushnoff, C. (2010). Diversity of antioxidant properties and phenolic content of date palm (*Phoenix dactylifera* L.) fruits as affected by cultivar and location. *Journal of Food, Agriculture and Environment*, 8, 253–260.
- Aluyor E.O., & Ori-Jesu, M. (2008). The use of antioxidants in vegetable oils – A review. *African Journal of Biotechnology*, 7, 4836–4842.
- Bassole, I.H., Ouattara, A.S., Nebie, R., Ouattara, C.A., Kabore, Z.I., & Traore, S.A. (2003). Chemical composition and antibacterial activities of the essential oils of *Lippia chevalieri* and *Lippia multiflora* from Burkina Faso. *Phytochemistry*, 62, 209–212.
- Capuano, E., & Fogliano, V. (2011). Acrylamide and 5-hydroxymethylfurfural (HMF): A review on metabolism, toxicity, occurrence in food and mitigation strategies. *Food Science and Technology*, 44, 793–810.
- Carelli, A.A., Franco, I.C., & Crapiste, G.H. (2005). Effectiveness of added natural antioxidants in sunflower oil. *Grasas y Aceites*, 4, 303–310.

- El-Rayes, D.A. (2009) Characterization of Three Date Palm Cultivars Based on RAPD Fingerprints and Fruit Chemical Composition. *Meteorology, Environment and Arid Land Agriculture, Journal of King Abdulaziz University*, 20, 3–20.
- Fernández-Bolaños, J.G., López, O., Fernández-Bolaños, J., & Rodríguez-Gutiérrez, G. (2008). Hydroxytyrosol and derivatives: isolation, synthesis, and biological properties. *Current Organic Chemistry*, 12, 442–463.
- Fernández-Bolaños, J., Rodríguez, G., Lama, A., & Sánchez, P. (2011). Dispositivo y procedimiento para el tratamiento de los subproductos de la obtención de aceite de oliva. National patent number: P201031236.
- Fuentes-Alventosa, J.M., Jaramillo-Carmona, S., Rodríguez-Gutiérrez, G., Guillén-Bejarano, R., Jiménez-Araujo, A., Fernández-Bolaños, J., & Rodríguez-Arcos, R. (2012). Preparation of bioactive extracts from asparagus by-product. *Food and Bioproducts Processing*, 9, 74–82.
- Gonçalves, B., Falco, V., Moutinho-Pereira, J., Bacelar, E., Peixoto, F., & Correia, C. (2009). Effects of elevated CO₂ on grapevine (*Vitis Vinifera* L.): Volatile composition, phenolic content, and in vitro antioxidant activity of red wine. *Journal of Agricultural and Food Chemistry*, 57, 265–273.
- Hammer, K.A., Carson, C.F., Dunstan, J.A., Hale, J., Lehman, H., Robinson, C.J., Prescott, S.L., & Riley, T.V. (2008). Antimicrobial and anti-inflammatory activity of five *Taxandria fragrans* oils in vitro. *Microbiology and Immunology*, 52, 522–530.
- Kostik, V., Memeti, S., & Bauer, B. (2013). Fatty acid composition of edible oils and fats. *Journal of Hygienic Engineering and Design*. 4, 112–116.
- Lee et al., 1989 – falta la referencia (se cita por el Rancimat method, p.12)

- Lisete-Torres, P., Losada-Barreiro, S., Albuquerque, H., Sánchez-Paz, V., Paiva-Martins, F., & Bravo-Díaz, C. (2012). Distribution of hydroxytyrosol and hydroxytyrosol acetate in olive oil emulsions and their antioxidant efficiency. *Journal of Agricultural and Food Chemistry*, 60, 7318–7325.
- Manzocco, L., Calligaris, S., Mastrocola, D., Nicoli, M.C., & Lericci, C.R. (2001). Review of non-enzymatic browning and antioxidant capacity in processed foods. *Trends in Food Science & Technology*, 11, 340–346.
- Mrabet, A., Jiménez-Araujo, A., Fernández-Bolaños, J., Rubio-Senent, F., Lama-Muñoz, A., Sindic, M., & Rodríguez-Gutiérrez, G. (2016). Antioxidant phenolic extracts obtained from secondary Tunisian date varieties (*Phoenix dactylifera* L.) by hydrothermal treatments. *Food Chemistry*, 1, 917–924.
- Mrabet, A., Rodríguez-Gutiérrez, G., Guillén-Bejerano, R., Rodríguez-Arcos, R., Ferchichi, A., Sindic, M., & Jiménez-Araujo, A. (2015). Valorization of Tunisian secondary date varieties (*Phoenix dactylifera* L.) by hydrothermal treatments: new fiber concentrates with antioxidant properties. *LWT - Food Science and Technology*, 60, 518–524.
- O'Sullivan, A., Mayr, A., Shaw, N.B., Murphy, S.C., & Kerry, J.P. (2005). Use of Natural Antioxidants to Stabilize Fish Oil Systems, *Journal of Aquatic Food Product Technology*, 14, 75–94.
- Park et al., 1999 – falta la referencia (se cita por el Rancimat method p.12)
- Putnik, P., Bursać Kovačević, D. & Dragović-Uzelac, V. (2016). Optimizing Acidity and Extraction Time for Polyphenolic Recovery and Antioxidant Capacity in Grape Pomace Skin Extracts with Response Surface Methodology Approach. *Journal of Food Processing and Preservation*, doi:10.1111/jfpp.12710.

- Rahman, N.A., & Salimon, J. (2006). Physicochemical characteristics of Terubok, Tenukuala fish oil. *Malaysian Journal of Analytical Sciences*, 10, 15–120.
- Rodríguez, R., Jaramillo, S., Rodríguez, G., Espejo, J.A., Guillén, R., Fernández-Bolaños, J., Heredia, A., & Jiménez, A. (2005). Antioxidant activity of ethanolic extracts from several asparagus cultivars. *Journal of Agricultural and Food Chemistry*, 53, 5212–5217.
- Rubio-Senent, F., Rodríguez-Gutiérrez, G., Lama-Muñoz, A., & Fernández-Bolaños, J. (2012). New phenolic compounds hydrothermally extracted from the olive oil by-product alperujo and their antioxidative activities. *Journal of Agricultural and Food Chemistry*, 60, 1175–1186.
- Rubio-Senent, F., Lama-Muñoz, A., Rodríguez-Gutiérrez, G., & Fernández-Bolaños, J. (2013). Isolation and identification of phenolic glucosides from thermally treated olive oil by-products. *Journal of Agricultural and Food Chemistry*, 61, 1235–1248.
- Rufian-Henares, J.A., & de la Cueva, S.P. (2009). Antimicrobial Activity of Melanoidins and Chelating Properties. *Journal of Agricultural and Food Chemistry*, 57, 432–438.
- Rufian-Henares, J.A., & de la Cueva, S.P. (2008). Assessment of hydroxymethylfurfural intake in the Spanish diet. *Food Additives and Contaminants*, 25, 1306–1312.
- Singleton, V.L., & Rossi, J.A. Jr. (1965). Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *American Journal of Enology and Viticulture*, 16, 144–158.
- Talibi, I., Amkraz, N., Askarne, L., Msanda, F., Saadi, B., Boudyach, E.H., Boubaker, H., Bouizgarne, B., & Ait Ben Aoumar, A. (2011). Antibacterial activity of moroccan plants

extracts against *Clavibacter michiganensis* subsp. *michiganensis*, the causal agent of tomatoes' bacterial canker. *Journal of Medicinal Plants Research*, 5, 4332–4338.

Xu, Q., Li, Y.H., & Lü, X.Y. (2007). Investigation on influencing factors of 5-HMF content in Schisandra. *Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)*, 8, 439–445.

Zhao, L., Chen, J., Su, J., Li, L., Hu, S., Li, B., Zhang, X., Xu, Z., & Chen, T. (2013). In vitro antioxidant and antiproliferative activities of 5-hydroxymethylfurfural. *Journal of Agricultural and Food Chemistry*, 6, 10604–10611.

Tables:

Table 1. Concentration of phenols and degradation products in the extracts obtained from thermally treated mixed dates. Antiradical activities of the two phenolic extracts.

	Steam treatment conditions	
	140 °C (PE140)	160 °C (PE160)
Volume of liquid fraction (L)	5.86	8.78
Phenolic extract (g extract/L liquid fraction)	2.99 ± 0.24*	10.02 ± 1.77*
Phenolic extract (g extract/Kg fresh date)	4.38 ± 0.35	21.98 ± 2.57
% Total phenols^a	11.63 ± 0.05*	8.78 ± 0.07*
<i>Phenolic compounds (mg/g of dry extract)</i>		
Gallic acid	16.565 ± 1.022	13.623 ± 0.814
Protocatechuic acid	2.831 ± 0.020*	1.153 ± 0.029*
Tyrosol	1.498 ± 0.044*	2.857 ± 0.062*
Vanillic acid	0.513 ± 0.012*	0.787 ± 0.010*
Syringic acid	0.479 ± 0.002	0.430 ± 0.011
p-coumaric acid	0.418 ± 0.019*	0.263 ± 0.009*
<i>Degradation products (mg/g of dry extract)</i>		
Hydroxymethylfurfural (HMF)	596.734 ± 25.308*	824.601 ± 40.040*
Furfural	0.052 ± 0.001*	0.118 ± 0.017*
<i>Antiradical activity</i>		
DPPH (EC ₅₀ mg/L)	2.798 ± 0.013	2.721 ± 0.022
TEAC (mmol Trolox [*] /g of extract)	0.062 ± 0.001	0.049 ± 0.001

^aTotal phenols were determined by Folin-Ciocalteu method and refer to dry extract.

* Significant difference ($p < 0.05$) between the two extracts.

Values shown are mean ± SD.

Table 2. Induction times (IT) and the percentage increase or decrease for the Rancimat tests (% Δ) in different vegetable and fish oils, using the two phenolic extracts. Values shown are mean $\pm$ SD.

Oils	Rancimat conditions (Temperature and air flow)	Thermal treatments							
		140 °C (PE140)					160 °C (PE160)		
		Control	Concentration of extract in oils (mg/L)						
				100	200	500	100	200	500
Olive oil	100 °C, 10 mL/min	IT (hours)	10.03 ± 0.64	10.95 ± 1.06	10.80 ± 0.17	11.71 ± 0.59	10.95 ± 0.63	10.09 ± 0.10	11.75 ± 0.36
		% Δ	-	9.15	7.61	16.72	9.13	8.73	17.13
Corn oil	80 °C, 10 mL/min	IT (hours)	9.04 ± 0.47	7.79 ± 0.47	8.71 ± 0.01	8.08 ± 0.04	8.27 ± 0.13	8.49 ± 0.09	9.93 ± 0.47
		% Δ	-	-13.79	-3.67	-10.67	-8.57	-6.11	9.84
Palm oil	100 °C, 10 mL/min	IT (hours)	35.33 ± 1.45	34.71 ± 1.56	33.49 ± 1.97	39.22 ± 1.19	34.70 ± 0.57	36.89 ± 1.07	39.80 ± 2.31
		% Δ	-	-1.77	-5.22	11.01	-1.79	4.4	12.65
Sunflower oil	100 °C, 10 mL/min	IT (hours)	1.97 ± 0.17	1.75 ± 0.06	1.72 ± 0.09	1.71 ± 0.02	10.68 ± 1.25	9.92 ± 0.59	8.85 ± 0.21
		% Δ	-	-11.17	-13.06	-13.22	440.94	402.78	348.32
Rapeseed oil	100 °C, 10 mL/min	IT (hours)	12.45 ± 0.29	12.30 ± 0.07	12.25 ± 0.42	12.32 ± 0.31	12.76 ± 0.15	13.11 ± 0.07	13.27 ± 0.14
		% Δ	-	-1.19	-1.59	-0.98	2.46	5.33	6.66
Fish oil	60 °C, 5mL/min	IT (hours)	12.05 ± 0.29	12.00 ± 0.30	11.37 ± 1.48	12.84 ± 0.45	11.90 ± 0.36	11.38 ± 0.80	13.08 ± 2.33
		% Δ	-	-0.44	-5.63	6.55	-1.28	-5.56	8.54

Table 3. Percentages of the increment of the induction times (% Δ) for the Rancimat tests in the different oils. Average and maximum values of % Δ obtained in Table 2. P/S and P+M/S ratios and the Pearson's Correlation Coefficients (R) between different treatments (average and maximum values of % Δ) and the ratios.

	Thermal treatment					
	140 °C		160 °C		P/S ratio	P+M/S ratio
	Average % Δ	max % Δ	Average % Δ	max % Δ		
Olive oil	11,16	16,72	11,66	17,13	0,49	5,90
Corn oil	-9,38	-3,67	-1,61	9,84	1,91	2,98
Palm oil	1,34	11,01	5,09	12,65	0,02	0,31
Sunflower oil	-12,48	-11,17	397,35	440,98	6,76	10,34
Rapeseed oil	-1,25	-0,98	4,82	6,66	3,32	14,90
Fish oil	0,16	6,55	0,57	8,54	1,05	1,67
R _{P/S ratio}	-0,74*	-0,88*	0,88*	0,88*	-	-
R _{P+M/S ratio}	-0,20	-0,48	0,38	0,37	-	-

*Significance level at P $\leq$ 0.05.

Table 4. Minimum inhibitory concentration (MIC) (mg/mL) of date extracts, PE140 and PE160, treated at 140 °C and 160 °C respectively, tested with different plant pathogenic bacteria. CECT, Spanish Type Culture Collection, strain catalogue number.

MICROORGANISM	MIC (mg/mL)	
	PE140	PE160
<i>Xanthomonas campestris</i> CECT 97	25.00	12.50
<i>Erwinia amylovora</i> CECT 222	6.25	3.13
<i>Erwinia toletana</i> CECT 5263	25.00	25.00
<i>Clavibacter michiganensis</i> subsp. <i>michiganensis</i> CECT 790	6.25	3.13
<i>Clavibacter michiganensis</i> subsp. <i>insidiosus</i> CECT 5042	25.00	12.50
<i>Rhizobium radiobacter</i> CECT 4119	25.00	12.50
<i>Pseudomonas savastanoi</i> CECT 5019	12.50	6.25
<i>Pseudomonas syringae</i> CECT 4429	12.50	6.25