

Enhancement of the antioxidant activity of a by-product (Phoenix dactylifera L.) from the Agri-food industry

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ARTICLE INFO

Article History: Received :29/01/2019

Accepted :09/12/2019

Key Words:

Date seeds, Reducing power, Antiradicalar activity, Flavonoids, Polyphenols.

ABSTRACT/RESUME

Abstract: Despite their biological value, date seeds as by-product are not quite studied, compared to analogue agricultural wastes. In addition to the basic physiochemical characterization, the antioxidant power (AP) of by-product (Phoenix dactyliferaL.) seeds (DS) is presently investigated. The AP was evaluated through the reducing power (RP), as well as antiradicalar activity (AA) of extracts obtained with different solvents (water, methanol, ethanol, acetone, chloroform, and hexane). Except hexane and chloroform, all other employed solvents give extracts with RP above 0.3 optic density units. Concerning the AA, all extracts obtained with cold extraction display a highest antioxidant activity for a dried extract concentration of 0.6 - 0.7 mg ml-1. In addition, the most important AA (> 90%) is found for BHT, vitamin E, ethanolic and methanolic extracts, whereas the least values (70-80%) report to the acetone and chloroform extracts. The similar results are also found when the hot extraction is applied. On the other hand, the most interesting correlations (R2>0.94) are reached for combinations of type polyphenols/RP (case of extraction with pure ethanol and 50% acetone), and flavonoids/RP (in the unique case of the extraction with 50%-acetone). Given their antioxidant potential, DS extracts can be recommended as an ingredient in various food and non-food formulations.

I. Introduction

Leaving aside local particularisms, the date palm (Phoenix dactylifera) fruit is for Saharan's what the olive (Oleaeuropea L.) fruit is for Mediterranean's. Algeria dwell on these both geographic areas and it is listed among the potential producers of dates in the world, with an annual production above 400000 tons, possessing moreover a considerable phytogenic diversity with more than 400 varieties (FAOSTAT, 2010).

The date fruit is above all appreciated for the sweetened and aromatized savor of its flesh. However, its industrial and domestic processing generates considerable amounts of wastes in form of seeds (Chandrasekaran&Bahkali, 2013). In their review about DS and its oil, Abdul Afiket al. (2013) deduced that the DS represent 11-18% (whole date weight), and its oil is provided with an antioxidant potential equivalent to that of olive oil. Numerous literature data are available about the AP

of seeds and stones of various plant species: citrus (Boccoet al., 1998), olives (Bouzideet al., 2005), pomegranate (Punica granatum L.) (Sadeghi et al., 2009), commonediblenut (Yang et al., 2009, noni (Morindacitrifolia) (West et al., 2011). However, few works are devoted to antioxidant properties of DS (Chairaet al., 2007; Al-Farsi & Lee, 2008; Ardekaniet al., 2010).Recently,Habibet al. (2013) have suggested the use of DS as nutraceutical and functional food ingredient, considering their phenolic composition. On the other hand, the world economic crisis of the last years renders indispensable the valorization of existing agroresources, in developing countries particularly (Ba et al., 2010).

The objective of the present work is to investigate the antioxidant activity of date seeds (DS) throughout the quantification of reducing power (RP) and antiradicalar activity (AA) at different conditions, namely: 1) the type of extraction solvent, and 2) the extraction procedure (hot and cold) applied in the case of AA measurement.

II. Materiel and methods

II.1. Partial physicochemical characterization of date seeds (DS)

The DS from Mech-Deglavariety were bought from the local market of Boudouaou in the suburbs of Algiers. Seeds were washed, dried at 50°C during 48 hours, and then grinded to obtain powder. Finally, the date seed powder (DSP) was kept at 4 °C up until analysis.

The basic characterization of DSP concerns the following indices: water content was determined by drying at 105± 2°C until constant weight, ash was determined by calcination at 550 ± 5 °C until whitish/greyish ash, titratable acidity (by 0.1N-NaOH solution in presence of phenolphthalein, the extract being obtained by mixing during 30 min, 1g of DSP with10 ml of freshly boiled distilled, followed by filtration), proteins was calculated by Kjeldhal method, fat was extracted by Soxhlet absorption atomic method, minerals by spectroscopy, sugars and carotenoids were quantified by the methodology described by Goodon, (1997) and Sass-Kiss et al. (2005).

All tests of characterization were repeated at least three times and the found results are expressed by the average value \pm standard deviation.

Fatty oil was extracted from 30g of DSP and the obtained oilwas thencharacterized for some parameters: acid index, acidity (oleic acid %), fatty acid %, peroxide indice, iodine indice, and insaponifiable fraction).

II.2. Polyphenols and reducing power

II.2.1. Preparation of extracts

9 solvents were used, either pure (distilled water, acetone, chloroform, ethanol, methanol and hexane) or diluted with distilled water (V/V ratio = 1/1): acetone/water, ethanol/water and methanol/water.

The extraction was carried out according to the procedure described by Al-Farsi and Lee (2008) with some modification. 1g of thin fraction of DSP was mixed (at $25\pm2^{\circ}$ C) with 250 ml of solvent sheltered from the light during 7, 14 and 30 days with regular stirring. The extract, enriched with

bioactive substances was then recovered by means of centrifugation 3000rpm/mn followed by filtration (Watman filter N°4). Finally, the extract was concentrated by reducing the volume of 50% at 50° C, using a rotary evaporator.

II.2.2. Total polyphenols

The content of total polyphenols of DS extracts were determined according to the colorimetric method of Folin-Ciocalteu (Yoo et al ., 2004). 1ml of each extract was mixed with 1 ml of Folin-Ciocalteu reagent; the reaction mixture was allowed to react for 5 min. 10ml of sodium carbonate 7% (w / v) are then added. The volume is made up with 25ml of distilled water. After 1 hour incubation in the dark and at room, the absorbance is read at 750 nm (UV / VIS spectrophotometer, Jasco V-530 Type). The results are expressed in terms of mg of gallic acid equivalents (GAE)/g DSP, using a calibration curve.

II.2.3. Total flavonoids

Total flavonoids in the extracts were determined according to the method reported by Kumazawa et al. (2004). Briefly,1 ml of extract was mixed with 1 ml of 2% aluminum chloride. The absorbance of the solution is measured at 420nm. The results are expressed in g of quercetin equivalent (QE) /g DSP, using a calibration curve.

II.2.4. Reducing power measurement

The RP of different DS extracts is made according to the procedure described by Oyaizu (1986). 2.5ml of extract were mixed with 2.5ml of sodium phosphate buffer (pH = 6.6) and 2.5ml of 1%potassium ferricyanide solution. After adding 2.5ml of trichloroacetic acid (10%), the mixture is incubated at 50 ° C for 20min and then it is centrifuged at 10000 rpm for 10 min. To 5ml of the supernatant are added 5 ml of distilled water and 1 ml of 0.1% ferric chloride. The absorbance of the solution is then measured at 700nm. The reducing power is expressed directly in units of optical density (absorbance).

II.2.5. Antiradicalar activity

II.2.5.1. Preparation of extracts

2 g of DSP are treated for 5 h with 250ml of four different solvents (chloroform, ethyl acetate, methanol and ethanol acetate) using Soxhlet apparatus. The filtered extract is then dried to dryness under vacuum at 40 $^{\circ}$ C using a rotavapor. Drying was completed in an oven at 40 $^{\circ}$ C until the constant weight was reached. The dry extract is dissolved in ethanol (reference solvent) and the resulting solution was stored at -4 $^{\circ}$ C until analysis. For comparison purposes, a cold extraction (maceration with magnetic stirring) is carried out by keeping unchanged the other operating conditions:

Sampling, solvent volume, duration and recovery mode of dry extract once the solvent is separated by sedimentation / filtration. The extraction yield (% of dry extract) is calculated as follows: Extraction rate = $100 \times (W1 - W0)/E$

where: W0 = weight of empty glass flask (g), W1 = weight of glass flask after evaporation (g), E : weight of the sample (2 g).

II.2.5.2. Antiradicalar activity (AA) measurement

After extraction, the quantification of AA is performed according to the protocol described by Bloise (1958) with some modifications. An aliquot of DSP extract is added to 2 ml of an ethanolic solution of 2-2-1-diphenyl picrylhydrazyl (DPPH) radical. The absorbance of the resulting mixture is measured at 517 nm after incubation in the dark for 30 min. AA is expressed in % of reduced DPPH:

 $AA = \% DPPH_{red} = [(Abs_{co} - Abs_{s}) / Abs_{c}] \times 100$

where $Abs_{co} = Absorbance$ of control, and $Abs_s = Absorbance$ of sample.

III. Results and discussion

III.1. Characterization of DS and their oil

It should be recalled that the characterization of DS (Figure 1A) is performed on the powder (Figure 1B). The latter was also used for the extraction of oil (DSO) (Figure 1C). The basic physicochemical characteristics of DS and DSO are given in Tables 1 and 2, respectively. All physicochemical indices are consistent with those of seeds from other varieties of date fruits reported by some authors: water content of Tunisian Deglet Nour and Allig varieties : 8.5-10 % (Besbes et al., 2004), ash : 0.8-1.16 % (Deglet Nour and Allig from Saudi Arabia) (Al- Farsi and al., 2007) , 0.96 and 1.0% (varieties of Oman and UAE) (Rahmanet al., 2004), fatty oil (hot extraction) : 10-13 % (Chaira et al., 2007).

Fatty oil, as a source of energy, especially plays an important role in the germination process of the seeds (Baud &Lepiniec, 2010). If we consider other kinds of fruit, the studied DS exhibit an oil content comprised in the range (11.6-14.5 %) revealed by Kamel&Kakuda (1992) who worked on kernels of kernels, cherries and plums. However, in the same study, these authors detect a concentration of only 0.6 % in the case of peach fruit. On the other hand, data of Table 1 show that the fat content found by the hot extraction is higher than that obtained with cold extraction. In the latter case, the yield of fat is comparable to results (8 to 12 %) communicated by Devshony et al. (1992) for other varieties of DS.



Fig 1. Date palm composition. A: date seed; B: date seed powder and C: date seed oil.

About DSO characteristics and taking into account the theme treated (antioxidant activity), it is primarily the two indices, iodine and peroxide, which require attention. Indeed, the iodine index is an indicator of the level of unsaturation, while the peroxide index is an expression of his damage state by oxidation. The iodine index of analyzed DSO is close to that (54.8 g iodine / 100 g) found by Besbeset al. (2005) on six Libyan date varieties. For cons, the value found is higher than that (50.92 g iodine / 100 g) advanced by Abdel- Nabey (1999) about six Egyptian date varieties. These authors communicate also a peroxide index (1.54 meg O2 / kg) close to that currently obtained. In all cases, the investigated DSO is conform to the standard of a refined oil (peroxide index <5), confirming thus its stability and resistance to oxidation.

 Table 1. Partial Physicochemical characteristics of date seeds.

Parameter	Mean value ± SD ,.g/100g,	
	unless otherwise stated	
Water content	7.87 ± 0.03	
Titratable acidity	3.20 ± 0.17	
Ash	1.21 ± 0.15	
Fat (hot extraction)	12.13 ± 1.27	
Fat (cold extraction)	10.13 ± 0.02	
Total sugars	3.94 ± 0.33	
Reducing sugars	3.42 ± 0.29	
Non reducing sugars	0.52 ± 0.03	
Carotenoïds (mg/100g)	99.00 ± 4.00	
Minerals (mg/100g) :		
Mg	42.50 ± 3.81	
Fe	5.13 ± 0.14	
Zn	3.11 ± 1.54	

Table 2. Basic	characteristics	of date	seed oil.
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Parameter	Mean value ± SD
Iodine index (g iodine /100g)	55.47±1.71
Acid index (g de KOH /g)	1.42±0.37
Acidity (% oleic acid)	0.71±0.11
Peroxide index (meq O ₂ / kg)	1.35 ± 0.06
Fatty acid (%)	75.78 ± 1.11
Insaponifiable value (%)	2.16±0.51

III.2. Polyphenols and flavonoids

Polyphenol and flavonoid total contents are illustrated by the histograms of Figures 2 and 3, respectively.

The highest rate (> 0.15 g GAE /g DSP) of polyphenol (Fig 2) is obtained with, pure methanol, 50% acetone, 50% methanol and 50% ethanol whatever duration extraction. But the maximum value (> 0.20 g GAE /g DSP) is given by the first three solvent after an extraction time of 30 days, the three results having no significant difference (p \leq 0.05). In opposite, the lower rates (<0.05 gGAE /g DSP) are obtained with hexane and chloroform which moreover do not present significant difference ($p \le 0.05$) for all the applied extraction times. The other three pure solvents (water, acetone and ethanol) gave intermediate concentrations of polyphenols and the nature of the solvent does not imply significant differences ($p \le 0.05$) in the values obtained for the same extraction time, except case of ethanol for a period extraction of 30 days.

These results show that the DS polyphenols are of different polarities. Furthermore, the concentrations are near (in the case of water) but higher (in the case of 50% acetone) to those found (~0.6 and 0.1g/g DS, respectively) by Al-Farsi and Lee (2008). In contrast, Al Farsi *et al.* (2007) found even lower results (3.1-4.43mg / g). These differences can be attributed to several factors, including the variety of fruit, methods of extraction and quantification, the level of sunshine...

The histogram related to the flavonoids (Figure 3) is similar to that of the polyphenols since the higher levels (> 0.1 QE /g DSP) of these substances are always obtained with the same solvents as above: 50 % ethanol, methanol, 50% methanol and 50 % acetone. Moreover, the values found for these four solvents do not present significant difference when the extraction is carried out for 30 days ($p \le 0.05$). Compared to their use in pure form, the solvents as 50%-aqueous solutions favor a better extraction of polyphenols and flavonoids which confirms the literature data (Yilmaz& Toledo, 2006). Apart from hexane and chloroform, all other extraction liquids result in flavonoids rate ranging from 0.04 to 0.14 g/g DSP. These results are similar to those (0.08-0.16g/ g DS) found by Al- Farsi et al. (2008) on the seeds from other types of date fruits but using solvents from the binary combinations of butanone, butanol, acetone and water. About other kinds of seeds, Bozanet al. (2008) reported significantly higher values (about 0.08 -0.18 g/g seeds) for 11 varieties of red grapes unlike polyphenols with values oscillating between 0.08 and 0.16g/g seed.



Fig 2. The influence of time and solvent on the flavonoids polyphenols extraction rate of the date seed powder (g / 100 g of extract). The identical alphabetical letters indicate the absence of a significant difference (p≤0.05) for each extraction times applied: 7 days (a, b, c, d), 14 days (a', b', c', d') and 30 days (a', b', c', d', e'').





Fig 3. The influence of time and solvent on the polyphenols extraction rate of the date seed powder (g / 100 g of extract).

The identical alphabetical letters indicate the absence of a significant difference ($p \le 0.05$) for each extraction times applied: 7 days (a, b, c, d), 14 days (a', b', c', d') and 30 days (a', b', c', d', e'').

III.3. Antioxidant power

It must be recalled that the AP is evaluated throughout RP and AA whose values are represented by the histograms of Figures 4 and 5, respectively

It is possible to clearly distinguish two groups of results for the RP (Figure 4): 1) reducing power almost zero (hexane and chloroform) and 2) 0.3 <RP<0.8 (all other solvents).

This result is consistent with the range (0.2-0.8) provided by Kchaou*et al.* (2013) about the pulp of six varieties of Tunisian dates when the extraction is performed with methanol / water (50/50, v / v). These authors found almost the same result by using the acetone / water mixture (70/30, v / v) with the exception of the *Bejo* variety for which RP = 1.24 OD units.



Fig 4. The absorbance of the powder extracts from the dates seed as a function of time and solvent (g / 100 g of extract).

The identical alphabetical letters indicate the absence of a significant difference ($p \le 0.05$) for each extraction times applied: 7 days (a, b, c, d), 14 days (a', b', c', d') and 30 days (a', b', c', d', e'').

These results prove that the date stones are rich in reducing substances of various chemical structures depending of their polarities. This result seems to be more reliable since the absorbance values found correspond to the domain of validity (0.3-0.7) of the Beer Lamber law. It is well known that the fruit kernels are provided with a high reducing power, compared to the edible part of the fruit. In this context, Stangeland*et al.* (2009) emphasize that the seeds of *Java* plum present a RP value of about 20 times greater than that of the pulp. Employing the

aqueous extracts of 28 fruits, Guoet al. (2003) found very varying results depending on the species: for example, the RP of seeds is more than 100 times that of the pulp in the case of red grapes and it is 5-10 times less than that of the pulp and skin in the case of date fruits. Allane and Benamara (2010) reveal that the seed RP of 12 seed fruits (including 4 varieties of dates) is sometimes 9 times higher, compared to other fruit tissues (skin and pulp).

Table 3 reports the correlations between levels of polyphenols and flavonoids on the one hand and the RP on the other hand. The most interesting correlations (R^2 > 0.94) are achieved for polyphenols / RP couples (case of extraction with pure ethanol and 50% acetone) and flavonoids / RP (only in the case of extraction with 50 % acetone).

Table 3. Correlation of type phenolic content/reducing powder (RP), and flavonoid content/RP, according to the type of extraction solvent.

Type of solvent	Polyphenols/RP	Flavonoids/RP
Distilled water	0.726	0.865
Ethanol	0.945	0.636
Methanol	0.923	0,033
Acetone	0.860	0.934
Hexane	0.181	0.592
Chloroform	0.872	0.877
Ethanol (50%)	0.673	0.680
Methanol (50%)	0.885	0.529
Acetone (50%)	0.985	0.943

extracts (> 50 %) are obtained with pure ethanol for both hot (HEL) and cold (CEL) extraction and for which there is no statistically significant difference ($p \le 0.05$) (Figure 5). From the results shown in Figures 6 and 7, it is easy to notice the similar shapes of the curves of variation of the AA as a function of the concentration of dry extract in the case of cold and hot extraction, respectively. In particular, it can be observed that there is a saturation concentration (~ 0.6mg.mL⁻¹) beyond which, AA remains almost constant. Moreover, for the both types of extraction (hot and cold), the highest AA (> 90%) are obtained with BHT, vitamin E, ethanol and methanol, whereas the lowest value (70-80%) corresponds to the solvent mixture ethyl acetate/chloroform.



Fig 5. Solids content of different types of solvents from ND/ ECNAE: hot extract of the nucleus by ethyl acetate, ECNCL: hot extract of the nucleus by chloroform, FNAE: cold extraction of the nucleus by ethyl acetate, EFNCL: cold extraction of the nucleus by the chloroform, EFNEL: cold extraction of the nucleus by ethanol, EFNML: cold extraction of the nucleus by methanol

The identical alphabetical letters indicate the absence of a significant difference ($p \le 0.05$) for each extraction solvants: 7 days (a, b, c, d), 14 days (a ', b', c ', d ') and 30 days (a' ', b' ', c' ', d'', e' ').



Fig 6. The percentage of free radical inhibition as a function of different concentrations of cold extract of the date seeds.

CEA = cold dry extract of the date seed with ethyl acetate, CCL= cold dry extract of the date seed with chloroform, CML = cold dry extract of the date seed with methanol, CCL= cold dry extract of the date seed with methanol.

CEL= cold dry extract of the date seed with l ethanol.





Fig 7. The percentage of inhibition of free radicals as a function of the different concentrations of hot extract of the date nucleus. HAE = hot dry extract of the date seed with ethyl acetate; HCL = hot dry extract of the date seed with chloroform; HML = hot dry extract of the date seed with methanol; HEL = hot dry extract of thedate seed with ethanol.

The AA of analyzed extracts is consistent with that given by Chairaet al. (2007) regarding the DS belonging to other Tunisian varieties, using as solvent chloroform and methanol but these authors found a higher value (average 51%) with ethyl acetate as solvent. Moreover, Chaalalet al. (2012) showed AA of 95% for fig seed extracts, using a hot extraction and acetone as an extraction solvent. As is apparent from this study, organic solvents are more effective for the extraction of antioxidant substances. In this context, Prasad et al. (2009) who worked on the lychee seed indicate that ethanolic and methanolic extracts have a radical scavenging activity greater than that of aqueous extract and BHT solution. For their part, Zhang et al. (2008) found that seeds of pomegranate display a scavenging activity lower than juice but the seeds possess a higher phenolic content. In practice, however, the regulation of dietary supplements recommended extraction with water or solvents with a very low alcohol fraction (Rolland, 2004).

IV. Conclusion

Results show that the date seeds are valuable in other forms than that of livestock feed. In particular, incorporation of their aqueous extract and oil in food formulations and cosmetics is possible. Based on its antioxidant composition, it is suggested to use date seed oil as edible cooking oil and also for the production of margarine due to the high stability and resistance of the oil to heat treatment which indicate good shelf life and storage.

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Please cite this Article as:

Lecheb F., Benamara S., Gougam H., Enhancement of the antioxidant activity of a by-product (Phoenix dactylifera L.) from the Agri-food industry, *Algerian J. Env. Sc. Technology*, 6:2 (2020) 1388-1395