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Optimization of Deglet-Nour Date *(Phoenix dactylifera L.)* Phenol Extraction Conditions

Lekbir Adel, Alloui-Lombarkia Ourida, Mekentichi Sihem, Noui Yassine, Baississe Salima

***Abstract*—**The objective of this study was to optimize the extraction conditions for phenolic compounds, total flavonoids, and antioxidant activity from Deglet-Nour variety. The extraction of active components from natural sources depends on different factors. The knowledge of the effects of different extraction parameters is useful for the optimization of the process, as well for the ability to predict the extraction yield. The effects of extraction variables, namely types of solvent (methanol, ethanol and acetone) and extraction time (1h, 6h, 12h and 24h) on phenolics extraction yield were evaluated. It has been shown that the time of extraction and types of solvent have a statistically significant influence on the extraction of phenolic compounds from Deglet-Nour variety. The optimised conditions yielded values of 80.19 ± 6.37 mg GAE/100 g FW for TPC, 2.34 ± 0.27 mg QE/100 g FW for TFC and 90.20 ± 1.29% for antioxidant activity were methanol solvent and 6 hours of time. According to the results obtained in this study, Deglet-Nour variety can be considered as a natural source of phenolic compounds with good antioxidant capacity.

***Keywords*—**Deglet-Nour variety, Date palm Fruit, Phenolic compounds, Total flavonoids, Antioxidant activity, Extraction, Optimization.

I. Introduction

DATE, fruit of the date palm (*Phoenix dactylifera* L.), very exploited in Mediterranean Africa, especially in the Algerian south, constitute an essential food for Muslims during all seasons, notably in the holy month of Ramadan.

Date palm fruits have been an important component of the diet in most of the arid and semiarid regions of the world. Several studies indicate that consumption of fruits and vegetables reduces the risk of several chronic diseases: coronary heart disease, blood pressure, obesity, diabetes and cancers [1], [2].

Dietary phenolic compounds and flavonoids have generally been considered, as non-nutrients and their possible beneficial effect on human health have only recently been recognized. These compounds are secondary metabolites that gather a large set of molecules, divided into fourteen chemical classes [3].

These valuable molecules possess very interesting biological properties, which are used in various fields, such as medicine, pharmacy and nutrition.

A. Lekbir, O. Alloui-Lombarkia, Y. Noui, and S. Baississe are with the Department of Food Technology, food science laboratory, University of Hadj Lakhdar-Batna, Algeria (e-mail: lekbiradel@yahoo.fr, ouridaa@hotmail.com, noui.yassine@gmail.com, baississe.nourhane@yahoo.fr).

S. Mekentichi was with the Department of Food Technology, food science laboratory, University of Hadj Lakhdar-Batna, Algeria (e-mail: mekentichisihem@yahoo.fr).

Extraction is an important step in the isolation and later in the identification and quantification of phenolic compounds; it is very difficult to develop a standardized extraction method that would simultaneously extract all phenolic compounds [4]. The extraction parameters may affect the quality and quantity of antioxidant activity. Many factors, such as solvent composition, the extraction time, temperature, pH, liquid-solid ratio and particle size, may significantly influenced the liquid-solid extraction. The extraction and purification of bioactive compounds from natural sources has become very important for the utilization of phytochemicals in the preparation of dietary supplements or nutraceuticals, functional food ingredients, and additives to food, pharmaceutical and cosmetic products [5].

The purpose of the present study was to examine the influence of extraction conditions (solvent type and extraction time) on extractability of phenolic compounds from Deglet-Nour variety, and to measure the antioxidant capacity of resultant extracts.

II. Material and Methods

A. Plant Material

The variety of date palm fruit used in this study is semi-soft date namely Deglet-Nour date (moisture content 26%). This variety was purchased from a local fruit supplier in Batna, Algeria. The samples were selected identically in terms of size, colour, ripening stage, without damaged and calamity, and were stored in paper bags at 4°C until use.

B. Chemicals and Standards

Acetone, ethanol, methanol, gallic acid, quercetin, Folin-Ciocalteu's reagent, sodium carbonate, aluminum chloride and DPPH (2,2-diphenyl-1-picryhydrazyl).

All chemicals were purchased from Sigma-Aldrich (USA), Fluka Chemie (Switzerland) and Merck (Germany). All Chemicals and reagents used were of analytical grade.

C. Moisture Content

Moisture was determined according to standard AOAC méthod 920.151 [6].

D. Extraction of the Phenolic Compounds

After cleaning and pitting dates, maceration was carried out on 1g of crashed pulp with 40ml of solvent at room temperature during different times with continued agitation. After centrifugation and filtration, the extracts were concentrated under reduced pressure at 40°C in a rotary evaporator. The extracts were kept in dark glass bottles inside the freezer until use. The storage conditions (time and

temperature) of extracts were the same for the all extraction conditions.

E. Experimental Design

In general, efficiency of the extraction of a compound is influenced by such multiple parameters as temperature, time and solvent type, and their effects may be either independent or interactive [7].

The experimental design for this study was performed to determine the appropriate range of conditions for phenolics extraction, namely, solvent type, and extraction time. In this study, effect of different solvents (methanol, ethanol and acetone) and different extraction times (1h, 6h, 12h and 24h) were investigated on the total phenolic extraction from Deglet-Nour date.

F. Total Phenolic Content

Total phenolics were determined using Folin-Ciocalteu's reagent as described by [8] with a little modification. Briefly, 0.5ml of each sample was mixed with 5ml of distilled water and 0.5ml of Folin-Ciocalteu's reagent, after 3min, 0.5ml of 7.5% sodium carbonate (Na_2CO_3) was added. The final mixture was shaken and then incubated for 1h in the dark at room temperature. The absorbance of all samples was measured at 760nm using Beckman 34 UV-Vis spectrophotometer. The total phenol concentration was calculated from the calibration curve, using gallic acid as a standard at concentrations ranging from 0 to 150µg/ml, and the results were expressed in mg of gallic acid equivalent (GAE) per 100g of fresh weight (fw).

G. Total Flavonoids

Total flavonoid content was determined using the colorimetric assay according to [9]. 1 ml of 2% aluminum methanolic trichloride solution ($AlCl_3$) was mixed with 1ml of the sample extracts. Test tubes were incubated at room temperature for 10min and the absorptance was determined at 415nm. The total flavonoids concentration was calculated from the calibration curve using quercetin as a standard (0-25 µg /ml), and the results were expressed as mg per 100g of fresh weight (fw).

H. Evaluation of Antioxidant Activity

In order to study the antioxidant activity of different extracts, we used the method based on the DPPH (2,2-diphenyl-1-picryhydrazyl) as a relatively stable radical, according to the protocol described by [10]. In this test, antioxidant s reduce the DPPH having a violet color into a yellow compound, the DPPH, of which the color intensity is inversely proportional to the capacity of antioxidant s present in the reaction medium to give protons [11]. 25 µl of sample were added to 975µl DPPH radical solution in solvent (6×10^{-5} M) and vortexed, the mixture was left in the dark during 30 min and the absorptance was read at 515nm.

The antiradicalaire activity is estimated according to the following equation [12]:

$$\% \text{ of antiradicalaire activity} = [(Abs_{515}\ DPPH - Abs_{515}\ Sample)/Abs_{515}\ DPPH] \times 100$$

where Abs_{515} DPPH is the absorbance of the control solution (containing only DPPH), and Abs_{515} Sample is the absorbance in the presence of the date extracts.

I. Statistical Analysis

Duncan's multiple range method and pearson's correlation were carried out for analyzing the experiment data, and to study the relationship between solvent type, extraction time, AA, TPC and TFC. Data were reported as means ± standard deviation of the mean of quadruple experiments. p values < 0.05 were regarded as significant and p values < 0.01 very significant. Data were analyzed using SPSS.

III. Results and Discussion

The aim of this study is to examine the influence of solvent types (acetone, ethanol and methanol) and time extraction (1, 6, 12 and 24h) on the extraction yield of phenolic compounds, flavonoids and antioxidant activity from Deglet-Nour date.

A. Total Phenolic Content

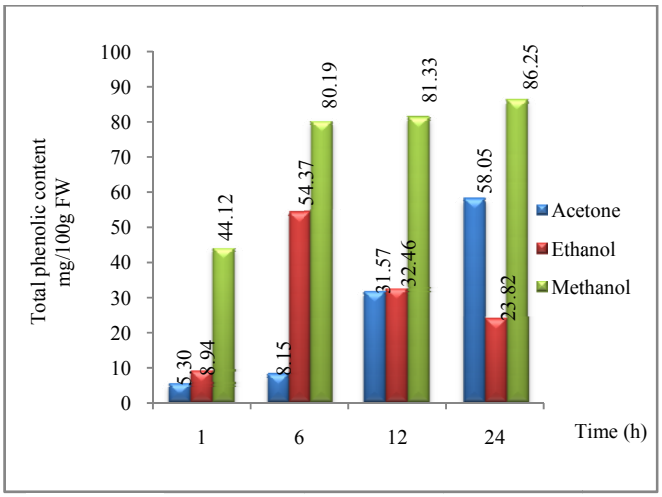

Fig. 1 Total phenolic content of different extracts

Experimental values of total phenolic content of different extracts were in the range from 5.30 to 86.25mg of gallic acid equivalents per 100g of fresh weight (fw).

Results of the total phenolic content showed that maximum phenolic content was obtained with methanol after 24h of extraction (86.25mg GAE/100g of fw) and the minimum phenolic content was obtained with acetone after 1h of extraction (5.3mg GAE/100g of fw).

B. Total Flavonoids

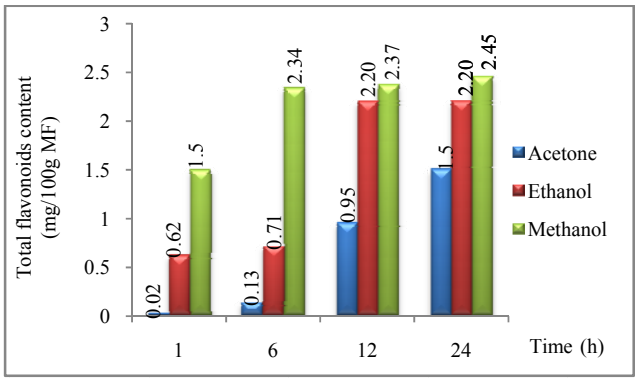

Fig. 2 Total flavonoid content of different extracts

Total flavonoid content of Deglet-Nour variety was measured using aluminum chloride colorimetric methods (Fig. 2). The results showed that the total flavonoid content of the different extracts varied considerably from 0.02 to 2.45mg in terms of quercetin equivalent/100 g fresh weight of sample.

Results of the total flavonoid content showed that maximum flavoinid content was obtained with methanol after 12h only of extraction (2.37mg QE/100g of fw) and the minimum flavonoid content was obtained with acetone after 1h of extraction (0.02mg QE/100 g of fw).

C. Antioxidant Activity

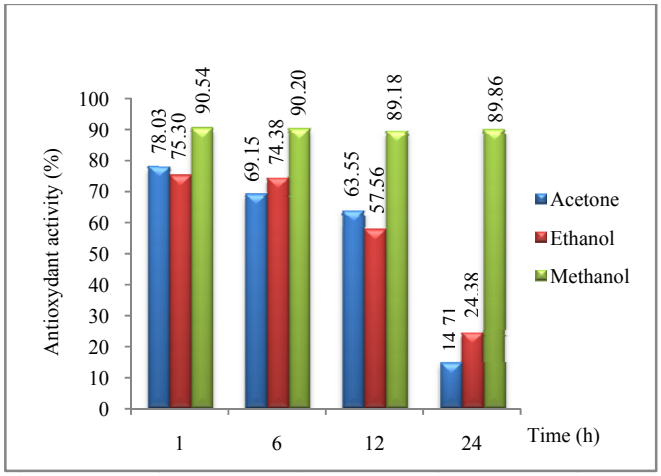

Fig. 3 Antioxidant Activity of different extracts

Activity is measured as the relative decrease in absorbance of DPPH as it reacts with the antioxidant (Rumbaoa, Cornago, & Geronimo, 2009). This method is widely used to evaluate antioxidant activity in foods.

The averages of antioxidant activity of different Deglet-Nour extracts based on DPPH assay are given in Fig. 3.

Antioxidant activity of the studied date variety was in the range of 14.71-90.54%. Methanol showed the highest level of antioxidant activity only after 1h of extraction (90.54%) and acetone exhibited the lowest level of antioxidant activity (14.71%).

D. Statistical Analysis

TABLE I
TOTAL PHENOLIC CONTENTS, TOTAL FLAVONOIDS AND THEIR ANTIOXIDANT ACTIVITY OF DIFFERENT EXTRACTS

Parameters	Polyphenols (mg/100g FW)	Flavonoids (mg/100g FW)	Antioxidant activity (%)
S1T2	5.30 ± 1.37^{a}	0.02 ± 0.29^{a}	14.71 ± 9.80^{a}
S1T1	8.15 ± 0.98^{a}	0.13 ± 0.02^{a}	78.03 ± 10.30^{e}
S2T1	8.94 ± 1.76^{a}	0.71 ± 0.10^{b}	57.56 ± 4.53^{c}
S2T3	23.82 ± 8.30^{b}	0.62 ± 0.31^{b}	75.30 ± 0.77^{e}
S1T3	31.57 ± 3.14^{c}	1.50 ± 0.18^{c}	63.55 ± 13.017^{cd}
S2T4	32.46 ± 4.53^{c}	2.20 ± 0.37^{d}	24.38 ± 2.28^{b}
S3T1	44.12 ± 2.33^{d}	1.50 ± 0.45^{c}	89.86 ± 1.10^{f}
S2T2	54.37 ± 5.42^{e}	2.20 ± 0.29^{d}	74.38 ± 1.55^{e}
S1T4	58.05 ± 6.64^{e}	0.95 ± 0.67^{b}	69.15 ± 3.18^{de}
S3T4	80.19 ± 8.91^{f}	2.45 ± 0.27^{d}	89.18 ± 2.58^{f}
S3T2	81.33 ± 6.37^{f}	2.34 ± 0.18^{d}	90.54 ± 1.29^{f}
S3T3	86.25 ± 5.59^{f}	2.37 ± 0.19^{d}	90.20 ± 2.20^{f}

(**Solvent**: **S1**: Acétone; **S2**: Ethanol; **S3**: Methanol. **Time**: **T1**: 1h; **T2**: 6h; **T3**: 12h; **T4**: 24h. **FW:** Fresh weight). Each value in the table is represented as mean ± SD (n = 4). Superscript letters with different letters in the same column, indicate significant difference ($P < 0.05$) analyzed by Duncan's multiple range test.

Total phenolic content was significantly influenced by solvent type and extraction time (Table I). Table I shows that total phenolic content of the extracts increased with increasing of extraction time. The optimum extraction conditions were found to be methanol solvent and extraction time of 6 h. Under the optimized conditions, the experimental value for TPC was 80.19mg GAE/100g FW.

Flavonoids are considering as phenolic compounds with highest antioxidant activity due to their chemical structure. Plant flavonoids are an important part of the diet because of their effect on human nutrition [5]. Results showed also that solvent type and time duration were the most factors affecting the TFC. Our research work confirmed that effect of different parameters on the yield of flavonoids was similar to the effects on the yield of phenolic content. The polarity of the solvent played an important role in the extraction of flavonoids.

The optimum extraction conditions were found to be methanol solvent and extraction time of 6h. Under the optimized conditions, the experimental value for TFC was 2.34mg QE/100g FW.

The optimal conditions for antioxidant activity were obtained after 1h of extraction for all solvents. Under these optimal conditions, the amount of antioxidant activity was 90.54%.

Nonetheless, results shows that there was no significant difference ($p > 0.05$) in antioxidant activity of methanolic extract with al extraction times.

E. Correlation

Pearson's correlation (Table II) only show a high positive significant relationship between antioxidant activity and phenol content ($r = 0.610$, $p < 0.01$). These results were in agreement with those reported by [10], [13] & [14]. However a low positive linear correlation was found between flavonoids ($r = 0.383$; $p < 0.01$) and antioxidant activity.

TABLE II
CORRELATION MATRIX BETWEEN ANTIOXIDANT ACTIVITY AND ANTIOXIDANTS

	AA
TPC	0.610**
TFC	0.383**

** $p < 0.01$

VI. CONCLUSION

Evaluation and optimization of phenolics extraction protocol are essential in order to maximize the extraction yield and to ensure accurate quantification of phenolic compounds in Deglet-Nour date. In the present study, methanol was indentified to be the best extracting solvent while it was predicted that the optimum extraction period was at about 6 h and prolonged extraction time was not useful because extraction was limited by solvent equilibrium.

Conclusively, the best solvent for the extraction of phenolic compounds in plant food depends very much on the variety of phenolic constituents in the food matrix. It is difficult to develop a general protocol for extraction of different phenolic acids from various matrices.

Thus, commonly used solvents should be evaluated in order to decide the most appropriate solvent for the optimum extraction of phenolics which in turn reflects the correct phenolic content of the tested sample.

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