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Phenolic profile and antioxidant activity of the Algerian ripe date palm fruit (*Phoenix dactylifera*)

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Abstract

The aim of this study was to determine the phenolic profile of seven different varieties of ripe date palm fruit (*Phoenix dactylifera*) from Algeria by LC–DAD–MS (ESI+), to investigate their respective antioxidant activities by the DPPH· method and to estimate their phenolic content using the Folin–Ciocalteu method. The total phenolic content was in the range of 2.49 ± 0.01 to 8.36 ± 0.60 mg gallic acid equivalents (GAE) per 100 g fresh fruit. This fruit was shown to possess an antioxidant activity, giving values of antiradical efficient (AE) from 0.08 ± 0.00 to 0.22 ± 0.00. The phenolic contents and the antiradical efficiencies of the different varieties were highly correlated ($R^2 = 0.975$). All the varieties were found to contain mainly *p*-coumaric, ferulic and sinapic acids and some cinnamic acid derivatives. Three different isomers of 5-*o*-caffeoylshikimic acid were detected. Different types of flavonoids were identified, mainly flavones, flavanones and flavonol glycosides.

Keywords: *Phoenix dactylifera*; Antioxidant activity; Phenolic profile; LC–MS

1. Introduction

The fruit of the date palm (*Phoenix dactylifera*) is considered to be an important fruit for the population living in the Algerian Sahara. It is considered a vital component of their daily diet. This fruit has great importance from nutritional and economic points of view.

Nowadays, the consumption of fruit and vegetables is regarded as important and good for health. Indeed, recent epidemiological studies have indicated that a high intake of fruit and vegetables is associated with reduced risk for a number of chronic diseases (Nicoli, Anese, & Parpinel, 1999). The recent explosion of interest in the bioactivity of the flavonoids of higher plants is due, at least in part, to the potential health benefits of these polyphenolic compounds as important dietary constituents (Rice-Evans, Miller, & Paganga, 1996). Thus, it is important to have a clear idea of the major phenolic families of which fruit and vegetables are comprised and the levels contained therein (Proteggente et al., 2002). The date palm fruit possesses antioxidant and antimutagenic properties in vitro (Vayalil, 2002). However, little is published on the phenolic profile of the ripe date fruit. Some authors (Lorente & Ferreres, 1988; Regnault-Roger, Hadidane, Biard, & Boukef, 1987) have presented a general view of the main phenolic compounds of date fruit, from Tunisia and Spain, respectively.

The aim of the present study is to explore the potential antioxidant activity, using the DPPH test, estimate the phenolic content using the Folin–Ciocalteu method and record the phenolic profile by the LC–DAD–MS technique, of seven different varieties of ripe Algerian date fruit.

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2. Materials and methods

2.1. Plant material

Seven different ripe date palm (*Phoenix dactylifera*) fruit varieties were harvested from Ghardaia (Algeria) and stored at −18 °C prior to analysis. The early variety Tazizaout was harvested at the end of August 2002, whereas the varieties Akerbouche, Deglet-Nour, Ougherouss, Tantbouchte, Tafiziouine and Tazerzait were harvested on October 15th, 2002. The different varieties were identified within the National Institute of Protection of Plants of Algeria (by Mr. Guendez E.).

2.2. Chemicals and standards

DPPH· (2,2-diphenyl-1-picryhydrazyl radical) and gallic acid (3,4,5-trihydroxybenzoic) were from Sigma, hydrochloric acid (minimum 37%); Folin–Ciocalteu's phenol reagent and acetic acid were from Merck; sodium chloride, methanol and sodium sulphate were from Readel-de Haën; diethyl ether (anhydrous) was from J.T. Baker; and Amberlite XAD-4 was from FLUKA.

2.3. Extraction of the phenolic fraction

Hundred grams of flesh and skin of date fruit were crushed in 300 ml methanol:water (4:1), then centrifuged (6000 rpm, 5 min). The supernatant was evaporated under vacuum at 40 °C. The cleanup was done according to the method prescribed by Tomás-Barberán, Isabel Martos, Ferreres, Radovic, and Anklam (2001) for honey. An XAD 4 resin glass column (4 cm, 15 cm height of resin) was prepared. It was washed with 200 ml of H_2O (pH 2 with 0.1 N HCl). The residue from the evaporation was treated with 200 ml of H_2O (pH 2 with 0.1 N HCl) and, after 5 min, the resulting mixture was passed through the column, washed with 100 ml H_2O (pH 2 with 0.1 N HCl) and 300 ml distilled water. Then, the phenolic fraction was eluted with 400 ml methanol. The methanol fraction was evaporated under vacuum at 40 °C. The residue was mixed with 10 ml brine and the phenolic fraction was extracted with 5 ml diethyl ether (three times). The ether extracts were combined and concentrated under vacuum at 30 °C. The residue was re-dissolved in 0.5 ml methanol. For HPLC analysis, the extracts were filtered on Gelman acrodisc filters (0.45 μm).

2.4. Estimation of the phenolic content by the Folin–Ciocalteu test

The total concentration of phenols in the extract was determined according to the Folin–Ciocalteu method (Waterman & Mole, 1994). In a 1.5 ml Eppendorf tube, 790 μl of distilled water, 10 μl of diluted sample (10^{-1}) and 50 μl of Folin–Ciocalteu reagent were added and vortexed. After 1 min, 150 μl of aqueous sodium carbonate (20%) was added, and the mixture was vortexed and allowed to stand at room temperature with exclusion of light, for 120 min. The absorbance was read at 750 nm, using an HP 8452A diode array spectrophotometer in a 10 mm cuvette. The total phenol concentration was calculated from the calibration curve, using gallic acid as a standard, and the results were expressed as mg of gallic acid equivalents (mg GAE) per 100 g fresh fruit.

2.5. Evaluation of antioxidant activity using the DPPH· method

The antioxidant activity was determined using the DPPH· test according to Brand-Williams, Cuvelier, and Berset (1995), Parejo, Codina, Petrakis, and Kefalas (2000) and Arnous, Makris, and Kefalas (2001). Different dilutions of the phenolic extract were prepared for each variety. An aliquot of 25 μl of diluted sample was added to 975 μl DPPH· solution (6×10^{-5} M) and vortexed. The decrease in the absorbance was determined at 515 nm when the reaction reached the plateau, using an HP 8452A diode array spectrophotometer in a 10 mm quartz cuvette. Methanol was used to zero the spectrophotometer. The absorbance of the DPPH· radical without sample was measured. The DPPH· concentration in the reaction medium was calculated from the calibration curve, as determined by linear regression:

$$A_{515nm} = 0.0262 \times [\text{DPPH}\cdot(\mu g/ml)] + 0.0068 \quad (R^2 = 0.999).$$

For each sample concentration tested, the percentage of DPPH· remaining, in the steady state, was calculated as follows:

$$\%\ \text{of remaining DPPH}\cdot = \frac{[\text{DPPH}\cdot]_{at:t=T}}{[\text{DPPH}\cdot]_{at:t=0}},$$

where T is the time necessary to reach the steady state.

The ratio [phenolic] (μg)/[DPPH·] (μg) was plotted against the % of remaining DPPH· to obtain the amount of sample necessary to decrease the initial DPPH· concentration by 50% (EC_{50}). The antiradical efficiency (AE) is calculated as follows:

$$AE = 1/EC_{50}.$$

2.6. Establishment of the phenolic profile using LC–DAD–MS (ESI+)

This analysis was performed using an LC/DAD/MS system with a Finnigan MAT Spectra System P4000 pump coupled with a UV6000LP diode array detector and a Finnigan AQA mass spectrometer. The separation was performed on a 125 × 2 mm Superspher 100-4 RP-

18 column (Macherey–Nagel, 4 μm particle size) at a flow rate of 0.33 ml/min and an injection volume of 1 μl. The detection was monitored at 290 and 340 nm and also by MS–ESI(+) spectroscopy at a probe temperature of 450 °C, probe voltage of 4.9 kV and at 20 and 100 eV in the mass analyser. The following gradient programme was used: (A) AcOH (2.5%) and (B) MeOH/AcOH (2.5%) (3:2), 70% A at 0 min, 60% A at 12 min, 40% A at 32 min and 20% A at 34 min. The data were processed using the Xcalibur 1.2 software.

3. Results and discussion

3.1. Antioxidant activity by the DPPH· method and total phenolic content

The different date fruit varieties presented a phenolic content in the range 2.49–8.36 mg/100 g fresh weight (Table 1). The Tantbouchte variety gave the highest value, followed by the variety Deglet-Nour, while the varieties Tazizaout and Ougherouss had the lowest values. These results showed that the date fruit has a low phenolic content compared with other fruits, such as strawberries (330 ± 4 mg/100 g FW), raspberries (228 ± 6 mg/100 g FW), apples (48 ± 1 mg/100 g FW) and tomato (30 ± 1 mg/100 g FW) (Proteggente et al., 2002).

The antioxidant activity was expressed by the parameter antiradical efficiency (AE) or antiradical power (ARP), where, the larger the ARP, the more efficient is the antioxidant (Brand-Williams et al., 1995).

Fig. 1 shows the kinetic behaviour of one variety (Deglet-Nour) at different dilutions, expressed as mass ratio (μg sample/μg DPPH·). The following general exponential model applies to all the varieties:

$$\ln[\%\ \text{of remaining DPPH}^{\cdot}] = a[\mu\text{g sample}/\mu\text{g DPPH}^{\cdot}] + b,$$

where "*a*" is the slope and "*b*" is the intercept (Brand-Williams et al., 1995; Parejo et al., 2000). High correlation coefficients were obtained. The higher the concentration, the steeper were the slopes and the lower the remaining DPPH· (Table1).

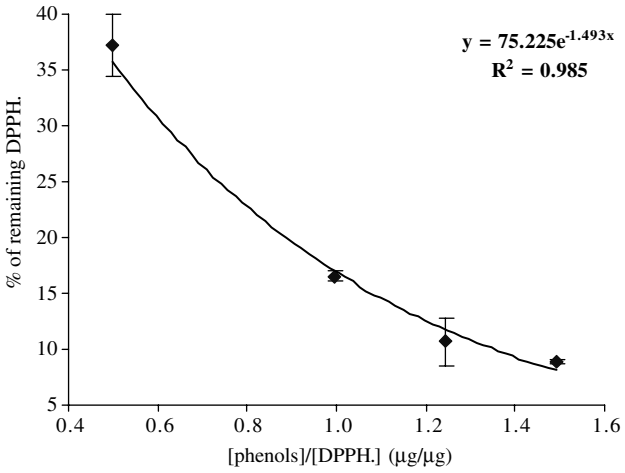

Fig. 1. Exponential curve of the percentage of remaining DPPH· as function of μg sample per μg DPPH· of the variety Deglet-Nour.

The Tantbouchte and Deglet-Nour varieties presented the highest values for AE and EC_{50}, respectively, whereas the varieties Tazizaout, Ougherouss and Akerbouche had the lowest values. A high correlation was found between total phenolic content and antiradical activity AE, $R^2 = 0.975$.

3.2. The phenolic profile using LC–DAD–MS (ESI+)

3.2.1. General profile

Under the conditions used, most of the compounds detected had intensive signals corresponding to the pseudo-molecular ion $[M+H]^+$. Formation of $[M+H_2O]^{+\cdot}$, $[M+Na^+]$ and $[M+CH_3OH]^{+\cdot}$ was observed as well. Adducts are expected in positive electrospray ionisation (ESI^+) (Kiehne & Engelhardt, 1996). The identification of the individual phenolic compounds was achieved by comparison of their UV–Vis absorption spectrum and MS data with the literature (Harborne &

Table 1
Efficient concentrations (EC_{50}), antiradical efficiencies (AE) and total phenolic contents of different date varieties

Variety name	EC_{50}[B]	AE[C]	Correlation coefficient (R^2)	Total phenolic content[A]
Tazizaout	12.7 ± 0.01a	0.08 ± 0.00a	0.99	2.49 ± 0.01a
Ougherouss	10.1 ± 0.12a,b	0.10 ± 0.00a,b	0.99	2.84 ± 0.41a,b
Akerbouche	10.2 ± 0.20a,b	0.10 ± 0.00a,b	0.99	3.55 ± 0.33b
Tazerzait	09.82 ± 0.84a,b	0.10 ± 0.01a,b	0.99	3.91 ± 0.39b,c
Tafiziouine	07.30 ± 0.12b	0.12 ± 0.01b	0.98	4.59 ± 0.43c
Deglet-Nour	06.09 ± 0.94c	0.17 ± 0.03c	0.99	6.73 ± 0.27d
Tantbouchte	04.55 ± 0.05d	0.22 ± 0.00d	0.98	8.36 ± 0.59e

Results are means ± SD ($n = 3$). Values of the same column, followed by the same letter, are not statistically different ($P < 0.05$) as measured by Duncan's test.

[A] mg gallic acid equivalents (GAE)/100 g fresh fruit (FW).

[B] Efficient concentration (μg sample/μg DPPH·): amount of antioxidant needed to decrease the initial DPPH· concentration by 50%.

[C] Antiradical efficiency: $1/EC_{50}$.

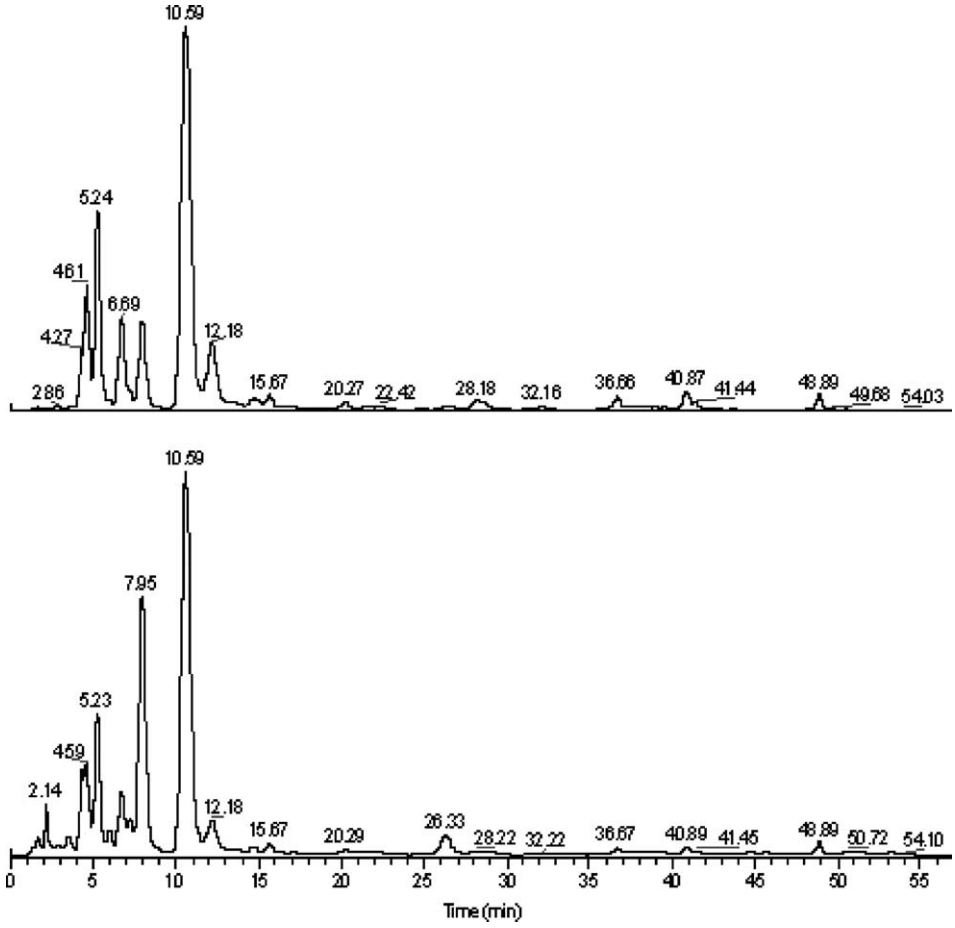

Fig. 2. HPLC chromatograms at 290 and 340 nm, respectively, of the variety Deglet-Nour.

Baxter, 1993; Hiroyuki, Honda, Nakagawa, Ashida, & Kanazawa, 2003; Long-Ze et al., 2000; Nianbai, Yu, & Prior, 2002). Fig. 2 shows the HPLC chromatogram of the variety Deglet-Nour at the wave lengths 290 and 340 nm. The MS and UV characteristics of the identified phenolics, for each variety, are given in Tables 2–8.

Throughout the obtained results, it appears that all the varieties contain more or less the same type of compounds with some slight differences.

3.2.2. Cinnamic acids

The most apparent compounds in all the varieties are the cinnamic acids and their derivatives. Ferulic, coumaric and sinapic acids are present in all the varieties as the major compounds. Coumaric acid is present in almost all varieties as *p*-coumaric acid, excepting the varieties Tazerzait, Tafiziouine and Tazizaout, where its derivatives were found.

Two cinnamic acid derivatives, with a molecular weight of 372, appear to be present in all varieties. The presence of the characteristic compound of the family of *Palmae* was also detected, which is 5-*o*-caffeoylshikimic acid (Harborne & Baxter, 1993) in all varieties, except for the variety Tazizaout. This compound was found in three isomeric forms with different retention times and fragmentation patterns. Another compound, xantoxylin, with a molecular weight of 196, was present in all varieties but Tazerzait and Ougherouss. Hydrocaffeic acid was detected in the varieties Tantbouchte, Tafizaouine, Tazerzait and Tazizaout. It should be mentioned, as well, that the varieties Deglet-Nour and Tazerzait were characterised by the presence of coumaroylquinic acid.

3.2.3. Flavonoids

3.2.3.1. General. Most of the flavonoids were flavones. Nevertheless, some flavonols and flavanones were detected. Compared with the cinnamic acids detected, the concentration of flavonoids was very low. The varieties Deglet-Nour, Tazizzout and Ougherouss seem to be the richest in flavonoids in terms of the number of different flavonoids detected. The variety Tazerzait is the poorest, where only three flavones were detected.

3.2.3.2. Flavones. The first flavone glycosides eluted have a molecular weight of 464 and 478, respectively. They

Table 2
Analysis of the HPLC and MS chromatograms for the variety Tantbouchte

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.43	169	–	147	246	Unknown compound
01.66	163	145	145	238, 290	Unknown compound
02.20	174	–	–	258, 294	Unknown compound
03.17	165	–	–	234, 298	Coumaric acid
03.63	395	199, 172, 131	199, 172, 131	254	Unknown compound
04.48	451	289, 234, 181, 163	–	262, 294	Caffeic acid derivative
04.94	337	163	163	242, 326	Three isomers of 5-*o*-caffeoylshikimic acid
05.61	337	163	181, 163	242, 326	
07.16	337	163	163	242, 326	
07.62	183	155	155	238, 310	Hydrocaffeic acid
08.26	165	147	147	238, 310	*p*-Coumaric acid
11.00	195	177	177	242, 318	Ferulic acid
12.67	225	207	207	242, 318	Sinapic acid
15.04	373	–	–	242, 286, 322	Cinnamic acid derivatives
16.00	373	–	–	242, 318	
20.50	465	303	303	258, 302, 318	Flavone glycoside
21.74	443	–	–	242, 310	Unknown compound
26.00	239	221	221	238, 298	Unknown compound
26.56	442	–	–	238, 278	Unknown compound
28.10	479	317	–	250, 330	Flavone glycoside
28.78	341	–	–	242, 286, 322	Flavone
32.33	565	–	–	239, 254, 258	Flavonol glycoside
36.70	607	–	–	246, 266, 318	Flavone glycoside
40.93	515	301	301	250, 330	Flavone glycoside
41.47	515	–	177	250, 322	Flavone glycoside
50.72	197	–	–	266	Xantoxylin

Table 3
Analysis of the HPLC and MS chromatograms for the variety Deglet-Nour

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.67	149	–	–	234, 262, 290	Unknown compound
02.13	339	136	136	258, 294	Coumaroylquinic acid
03.42	172	–	–	254	Unknown compound
04.27	473	197	197	262, 294, 318	Unknown compound
04.59	337	163	163	242, 326	5-*o*-Caffeoylshikimic acid isomers
05.23	337	–	163, 181, 157	242, 322	
06.69	337	163	163	242, 326	
07.95	165	147	147	238, 306	*p*-Coumaric acid
10.59	195	177	177	242, 314, 330	Ferulic acid
12.18	225	207	207	242, 318	Sinapic acid
14.70	373	164	164	242, 286, 322	Cinnamic acid derivatives
15.67	373	–	–	246, 322	
20.29	465	303	303	238, 254, 322	Flavone glycoside
21.57	443	221, 189	221, 189	242, 310	Unknown compound
22.42	219	–	–	242, 278, 338	Unknown compound
28.10	479	317	317	254, 354	Flavonol glycoside
28.58	479	317	317	238, 330	Flavone glycoside
32.22	565	–	–	254, 294, 358	Flavonol glycoside
36.66	607	287	287	246, 266, 342	Flavone glycoside
40.87	515	331, 301	331, 301, 203, 177	238, 266, 338	Flavone glycoside
41.44	515	–	177	246, 326	Flavone glycoside
48.89	413	351, 331, 309, 291	351, 331, 291, 171	238, 314	Unknown compound
50.72	197	–	–	266	Xantoxylin

Table 4
Analysis of the HPLC and MS chromatograms for the variety Ougherouss

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.43	164	–	147	242	Unknown compound
02.20	174	–	–	258, 294	Unknown compound
03.12	370	165, 147	165, 147	238, 306	Coumaric acid derivative
3.61	194	–	–	254	Unknown compound
04.51	194	–	–	266, 290	Unknown compound
04.95	337	163	163	242, 326	5-*o*-Caffeoylshikimic acid isomers
05.61	337	163	163	242, 330	
06.25	153	–	–	282, 310, 238	Gallic acid derivative
07.15	337	252,163	252, 163	242, 326	5-*o*-Caffeoylshikimic acid isomer
08.25	165	147	147	238, 306	*p*-Coumaric acid
11.00	195	177	177	242, 318	Ferulic acid
12.67	225	207	207	246, 326	Sinapic acid
15.02	373	–	–	242, 322	Cinnamic acid derivatives
15.94	373	–	–	246, 322	
20.52	465	303	303	254, 338	Flavone glycoside
21.75	443	–	–	242, 310	Unknown compound
26.54	551	287	287	266, sh 325	Flavanone glycoside
28.35	479	317	317	254, 346	Flavone glycoside
28.72	373	137	137	242, 286, 326	Unknown compound
29.95	501	419, 401, 167	419, 401, 208, 167	242, 270, 350	Flavone glycoside
32.29	565	317	–	254, 298, 354	Flavone glycoside
36.71	607	287	287	270, 330	Flavone glycoside
40.91	515	301	301,177	254, 266, 334	Flavone glycoside
41.45	515	177	343, 177	250, 322	Flavone glycoside

Table 5
Analysis of the HPLC and MS chromatograms for the variety Tafiziouine

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.43	365	210, 164	210, 164	242	Unknown compound
01.65	164	148	148	238, 294	Unknown compound
02.22	174	–	–	258, 294	Unknown compound
03.59	172	–	–	254	Unknown compound
04.88	337	–	–	242, 298, 330	5-*o*-Caffeoylshikimic acid isomers
05.50	337	–	–	242, 330	
06.90	337	163, 252	163, 252	246, 330	
07.30	183	–	–	238, 306	Hydrocaffeic acid
08.16	206	197, 165, 147	197, 165, 147	238, 310	Coumaric acid derivative
10.58	195	177	177	242, 318	Ferulic acid
11.91	225	207	207	242, 381	Sinapic acid
14.18	373	–	–	238, 322	Cinnamic acid derivatives
15.18	373	–	–	242, 286, 322	
20.74	443	–	–	242, 310	
24.02	443	–	–	238, 318	Unknown compound
25.51	551	282	282	270, sh 320	Flavanone glycoside
27.69	373	–	–	242, 330	Unknown compound
31.17	565	–	–	254, 350	Flavone glycoside
36.25	607	277	–	242, 318	Flavone glycoside
40.77	515	–	177	238, 318	Cinnamic acids derivative
41.30	515	–	177	250, 322	Flavone glycoside
43.53	515	–	177	254, 290, 318	Flavone glycoside
50.40	197	–	–	266	Xantoxylin

Table 6
Analysis of the HPLC and MS chromatograms for the variety Tazerzait

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.45	315	229, 210, 164	164, 147	246	Unknown compound
01.66	339	220	136	234, 294	Coumaroylquinic acid
03.23	304	165	165, 147	234, 298	Coumaric acid derivative
03.70	237	209, 194, 172	209, 194, 172	254	Unknown compound
04.69	473	445, 212, 178, 234	445, 234, 212, 178, 161	234, 262, 290	Unknown compound
05.27	337	–	163	242, 326	5-*o*-Caffeoylshikimic acid isomer
05.53	227	197	197, 143	234, 274	Unknown compound
05.93	337	–	–	242, 330	Dactyliferic acid isomer
06.47	227	–	153	238, 282, 310	Unknown compound
07.82	183	–	–	238, 306	Hydrocaffeic acid
08.66	165	147	147	238, 310	*p*-Coumaric acid
11.41	195	177	177	242, 318	Ferulic acid
13.06	225	207	207	242, 318	Sinapic acid
15.18	373	–	–	242, 322	Cinnamic acid derivatives
16.11	373	–	–	242, 322	
21.75	443	–	–	238, 310	Unknown compound
26.03	239	221	221	238, 302	Unknown compound
28.81	373	343	137	242, 326	Cinnamic acid derivative
36.72	607	387	–	238, 270, 314	Flavone glycoside
40.94	515	301	301	250, 326	Flavone glycoside
41.47	515	–	343, 177	250, 322	Flavone glycoside
48.91	291	–	–	314	Unknown compound
50.60	197	–	–	266	Xantoxylin
53.30	497	293, 333	333, 293	274	Unknown compound

Table 7
Analysis of the HPLC and MS chromatograms for the variety Akerbouche

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.70	145	–	–	238, 294	Unknown compound
02.23	174	–	–	258, 294	Unknown compound
04.56	291	234, 194, 181, 166	234, 194, 181, 166	294	Unknown compound
05.08	337	163	163	242, 326	5-*o*-Caffeoylshikimic acid isomers
05.77	337	181, 163	181, 163	242, 326	
07.34	337	163	163	242, 326	
08.38	165	147	147	238, 310	*p*-Coumaric acid
11.18	195	177	177	242, 318	Ferulic acid
12.90	225	207	207	242, 322	Sinapic acid
15.20	373	–	–	242, 322	Cinnamic acid derivatives
16.08	373	–	–	246, 322	
20.55	465	303	303	266, 302, 318	Flavone glycoside
21.73	443	209	209	242, 310	Unknown compound
26.02	239	221, 201	221, 201	238, 302	Unknown compound
26.50	551	443, 287, 265, 247	443, 303, 283, 247	270, sh 325	Flavanone glycoside
28.37	479	317	–	254, 342	Flavone glycoside
28.75	373	–	–	242, 322	Unknown compound
32.30	565	–	–	254, 298, 350	Flavonol glycoside
36.70	607	287	287	242, 266, 326	Flavone glycoside
40.92	515	301	301, 177	254, 330	Flavone glycoside
48.93	291	149	–	314	Unknown compound
50.69	197	–	–	266	Xantoxylin

Table 8
Analysis of the HPLC and MS chromatograms for the variety Tazizaout

R.T. (min)	$[M+H]^+$ (m/z)	Fragment ion (m/z) at 20 eV	Fragment ion (m/z) at 100 eV	λ_{max} (nm)	Identification
01.43	164	–	–	246	
03.55	194	172, 167, 163	172, 167, 163	254	Unknown compounds
04.36	344	193, 178	193, 178	262, 290	
05.15	197	–	–	234, 274	Unknown compound
06.04	227	–	–	238, 282, 306	Unknown compound
07.22	183	–	–	238, 306	Hydrocaffeic acid
07.96	465	447, 208, 197, 165, 147	447, 208, 197, 165, 147	238, 306	Coumaric acid derivative
10.40	195	177	177	242, 318	Ferulic acid
11.78	225	207	207	242, 318	Sinapic acid
14.15	373	–	–	242, 322	Cinnamic acid derivatives
15.16	373	–	–	242, 322	
19.62	465	229	229	242, 306, sh 365	Flavanone glycoside
20.72	403	–	–	242, 310	Unknown compound
21.49	447	401	439	246, 338	Flavone glycoside
23.86	419	–	–	242, 318	Flavone glycoside
24.84	239	–	–	238, 302	Unknown compound
25.77	449	–	–	234, 278, sh 330	Flavanone glycoside
27.41	373	–	137	242, 326	Cinnamic acid derivative
29.10	501	271	–	238, 330	Flavone glycoside
29.57	447	–	–	238, 342	Flavone glycoside
30.75	565	–	–	250, 358	Flavonol glycoside
31.27	273	144	144	234, 290, sh 335	Flavanone
35.93	607	287	287	246, 318	Flavone glycoside
36.72	401	193, 175	–	242, 310	Unknown compound
37.66	223	177	177	246, 310	Ferulic acid derivative
39.95	301	–	203, 177	250, 342	Flavonol
49.25	197	–	–	266	Xantoxylin
51.02	219	145	145	278	Unknown compound

were detected within the varieties Akerbouche, Tantbouchte, Deglet-Nour and Ougherouss. Three other flavone glycosides were detected within all varieties. They have molecular weights of 564, 606 and 514. Another flavone glycoside with a molecular weight of 514 was detected within the varieties Tazerzait, Tafiziouine, Tantbouchte, Deglet-Nour and Ougherouss. The variety Tazizaout showed the presence of four other flavone glycosides with molecular weights of 446,418, 500 and 447.

3.2.3.3. Flavanones. The flavanone glycosides were not so numerous. Thus, only the varieties Tafiziouine, Akerbouche and Ougherouss each presented a different flavanone glycoside, while the variety Tazizaout had three different flavanone glycosides. The different flavanone glycosides detected within the varieties Tafiziouine, Akerbouche and Ougherouss had the same molecular weight of 550, but the fragments were different from one variety to another. The two flavanone glycosides of the variety Tazizaout have molecular weights of 464 and 448. The first one has a fragment of 229, while the second one did not show fragmentation. The third flavone detected had a molecular weight of 272.

3.2.3.4. Flavonols. As for the flavanones, the flavonols were not so numerous as the flavones. Thus, only one flavonol glycoside was found for each variety of Tantbouchte, Akerbouche, and Tazizaout, while two flavonol glycosides were detected for the variety Deglet-Nour. The variety Tazizaout was characterised, also, by the presence of a non-glycosylated flavonol. A flavonol glycoside with a molecular weight of 565 was present in all the varieties cited above. This flavonol glycoside did not show any fragmentation. The other flavonol glycoside had a molecular weight of 478, which is suggestive of methylquercetin glycoside; it was detected within the variety Deglet-Nour. The non-glycosylated flavonol had a molecular weight of 300.

From the obtained results, it seems that the date fruit possesses a potential antioxidant activity, even though the phenolic content, estimated by the Folin–Ciocalteu method, was not high compared with other species of fruits. The HPLC–DAD–MS analysis showed that the major phenolic compounds of the date fruit are cinnamic acids. Ferulic, sinapic and coumaric acids and their derivatives, such as 5-*o*-caffeoylshikimic acid, are the dominant ones. This high content of free cinnamic acid is not frequently encountered in fruits. According

to Macheix, Fleuriet, and Billot (1990), the presence of free forms of hydroxycinnamic acids can result from the intense extraction conditions, where high temperature and high or medium acid media can lead to partial hydrolysis of the combined forms. Nevertheless, according to Regnault-Roger, Hadidane, Biard, and Boukef (1986), only date fruit seems to display high free ferulic and *p*-coumaric acid contents. Macheix et al. (1990) reported that this high free hydroxycinnamic acid content is probably associated with the original maturation of the fruit in which the browning which occurs is certainly due to sub-cellular compartmentation, as is the hydrolysis of the combined forms of hydroxycinnamic acids. The diversity of hydroxycinnamic acids encountered in plants, and particularly in fruits, thus results from the nature of the bonds and that of the molecules involved (Macheix et al., 1990; Molgaard & Ravn, 1988). The presence of a double bond in the lateral chain of these compounds leads to the possible existence of two *cis* and *trans* isomeric forms. Interco-version of the two forms might occur in situ and be responsible for certain physiological responses in plants (Towers & Yamamoto, 1985). This conversion takes place very easily under the effect of light (Kahnt, 1967) and, in particular, during extraction and purification operations prior to analysis. The presence of 5-*o*-caffeoylshikimic acid (dactyliferic acid) was also observed, which is a shikimic ester. According to Harborne, Williams, Greenham, and Moyna (1974), this compound is a contributor to the browning reactions which take place during the maturation of the fruit. The results from the present study also showed the presence of certain flavonoids, mainly flavone glycosides, flavonone glycosides and flavonol glycosides. But the concentrations of these compounds were too low and their identities cannot be clearly established. Concerning the antioxidant activity, a high correlation between the phenolic content and the antiradical efficiency was found. The HPLC–DAD–MS showed that the cinnamic acids were the dominant compounds in the phenolic profile of the date fruit. The presence of $-CH=\overset{|}{C}-COOH$ groups in cinnamic acids ensures greater H-donating ability and subsequent radical stabilisation than the carboxylic group in benzoic acids. The reduction potentials of radicals derived from 3,4-dihydroxybenzoate derivatives decrease with the electron-donating power at C1. Thus, caffeic, sinapic, ferulic and *p*-coumaric acids were found to be more active than protocatechuic, syringic, vanillic and *p*-hydroxybenzoic (Cuvelier, Richard, & Bercet, 1992). Since the phenolic profile of date fruit is mainly constituted of ferulic, sinapic and *p*-coumaric acids, this potential antioxidant activity was expected.

Finally, this phenolic profile could serve as a tool to justify the geographic origin of these date fruit varieties. Nevertheless, further studies are needed to characterise the main flavonoids, using LC–MS with other methods of isolation.

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