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Valorisation of Clementine peels for the recovery of minerals and antioxidants: Evaluation and characterisation by LC-DAD-MS of solvent extracts

[1*]Boudries, H., [3]Souagui, S., [1]Nabet, N., [3]Ydjedd, S., [2]Kefalas, P., [1]Madani, K. and [1]Chibane, M.

[1]Laboratory of Biomathematics, Biochemistry, Biophysics and Scientometrics, Faculty of Nature and Life Sciences, University of Bejaia, 06000 Bejaia, Algeria
[2]Department of Food Quality and Chemistry of Natural Products, Mediterranean Agronomic Institute of Chania/ Centre International de Hautes Etudes Agronomiques Méditerranéennes, PO Box 85, 73100 Chania, Crete, Greece
[3]Faculté des Sciences de la Nature et de la Vie, Université de Bejaia, 06000 Bejaia, Algérie

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Abstract

Six peel samples of Clementine fruits and one sample of Mandarin cultivar peel were studied. Mineral analysis showed that all the fruit peels were good sources of K, Ca, Na and Fe. Phenolic and flavonoid contents presented values between 9686±144 and 11934±312 mg gallic acid equivalents /100 g dw and between 702±68 and 1047±54 mg Catechin equivalents /100 g dw respectively, and in both cases the highest amount was found in Cadoux cv. and the lowest in St Martin cv. whereas for carotenoid contents, the amount varied from 52±1 to 76±1 mg β-carotene equivalents /100g dw and Rocamora cv. was the richest fruit. The LC-DAD-MS analysis of phenolic compounds showed that hesperidin was the major flavonoid, and for the first time natsudaidain derivative is reported. All Clementine peels exhibited DPPH scavenging activity and reducing power, the Cadoux cv. being the most active one while Rocamora and Merme cv. were the weakest in both tests, respectively.

Introduction

The domestic and agricultural products processing industries produce substantial quantities of by-products, which are currently generally treated as waste of juice industry, this practice is not only a waste of resource, but also causes environmental pollution and they gradually ferment and release off odours. Interestingly, the peel of citrus fruits may be rich sources of natural, low-cost antioxidants and have higher antioxidant activity than the pulp fractions (Guimarães *et al*., 2010; Goulas and Manganaris, 2012; Lagha-Benamrouche and Madani, 2013).

Citrus is an important crop, available throughout the year especially during the peak season of October to May, mainly used in food industries for fresh juice production. Citrus peel, which is the main by-product represents roughly half of the fruit mass and is a rich source of bioactive compounds (Li *et al*., 2006), remains for the major part unutilized. The valorization of these byproducts as sources of natural antioxidants to be used in food applications remains a challenge.

Clementine *(C. Clementina)* is a hybrid between orange and mandarin, discovered by Father Clement Rodier in Misserghin, near Oran, in Algeria (Khan, 2007) and various Clementine clones are commercially very important in Mediterranean and North African countries. In Algeria, Clementine fruit production comes in the second position after oranges. In 2012, Clementine production represented 15.70% of total citrus fruit (Agriculture Ministry Statistics, 2012), which reflects the preference of Algerian population for this species, which has a typical sweet taste, bright orange colour and easy for consumption.

So far, Clementine peels have been rarely investigated, and limited data are available on the phenolic composition of the peel, and especially of Clementine cultivars. Furthermore, several minerals are well known, as nutritionally essential, others are considered toxic; presently, little is known about the levels of minerals in citrus peels. As part of our ongoing research on antioxidants from natural resources, we developed herein a comparative study, among six Clementine cultivar peels, cultivated in Algeria and in order to determine for the first time (I) the mineral content (II) the phenolic and carotenoid contents, (III) the antioxidant activities using the DPPH method and reducing power and (IV) in order to study their phenolic profile by LC-DAD-MS.

*Corresponding author.
Email: *boudries.hafid@gmail.com*

Materials and Methods

Fruit and sample preparation

Six cultivars of Clementine fruits, belonging to the Mandarin group and can be distinguished from Mandarin by a rather deep colour of the rind and its sweet taste, were harvested at a commercial maturity stage, based on size uniformity and external colour, from a local farm of ITAF (Technical Institute of Fruit Arboriculture and Vine) on the 10th of December, 2008 in the region of Blida. Also, one cultivar of Mandarin *(C. reticulata)* was collected from Bejaia; both regions are located in north Algeria with Mediterranean climate.

Immediately after harvesting, fruits were washed with distilled water to remove superficial contamination, and then separated into edible and inedible portions. Peels were removed manually and were subsequently cut into small pieces and oven dried at 40°C during 3 days, then ground into a fine powder using a homogenizer. To achieve a standard size of particles, all the ground material was sieved through a 0.5 mm metal sieve and stored at -20°C until used.

Mineral contents of Clementine and Mandarin peels

The mineral contents of citrus dried peels were determined according to (Leterme *et al.*, 2006). The samples (2 g) placed in platinum crucibles were ashed in a muffle furnace at a temperature of 450 ± 10°C for 6 h; the ash was then weighed and digested with 5 mL of HNO_3(65%)/ $HClO_4$(72%) (2/1), the solution was filtered into a 100 mL flask, concentrated HNO_3 was added and the resulting solution was heated on a hot plate, then diluted to volume with deionized water. Sodium (Na), Potassium (k), Calcium (Ca), Iron (Fe), Zinc (Zn), Manganese (Mn), Copper (Cu) and Magnesium (Mg) were determined using an Atomic Absorption Spectrophotometer AAS (AA-6501F. Ver 1.10 Shimadzu Corporation). The amounts of minerals were calculated against standard curves.

Determination of carotenoids

150 mg of dried peel powder was extracted with 50 ml of (hexane, acetone, ethanol, 50:25:25. v/v/v) containing 0.01% BHT with a mortar and pestle until the extract were colourless, the top coloured layer of hexane was recovered and transferred to a 25 mL volumetric flask. The combined hexane phases were centrifuged for 5 min at 6500 rpm at 5°C. The content of carotenoids was determined by the measurement of the hexane extract absorbance at 450 nm. The result was expressed as mg of β-carotene per 100 g of dried peel (Sass-Kiss *et al*., 2005).

Extraction of phenolic compounds

Approximately 0.4 g aliquot of dried samples and 10 mL of 1% HCl in 80% aqueous methanol were stirred at room temperature for 3 h. The resulting mixture was then centrifuged at 4000 rpm for 20 min at 4°C. The pellet was re-extracted three times more by repeating the same steps under the same conditions. Following centrifugation the supernatants were combined and filtered through Whatman filter paper (Wang *et al*., 2006).

Total flavonoid contents

The total flavonoid content of the samples was measured by the colorimetric method of Marinova *et al.* (2005). Properly diluted peel extract (1 mL), was added to 4 ml of distilled water; followed by 0.3 mL of 5% $NaNO_2$ and the mixture was kept for 6 min at room temperature. 0.3 mL of 10% $AlCl_3$ methanol solution were added, and the mixture was incubated for 6 min again. After 5 min, 2 mL of 1 N NaOH were added and the total volume was made up to 10 mL with distilled water, the absorbance was measured at 510 nm after incubation for 10 min at room temperature against prepared reagent blank. The total flavonoid content was expressed as Catechin Equivalents (CE) per 100 g of dried peel using a calibration curve.

Total phenolic contents

The concentration of total phenols was measured by the Folin-Ciocalteu reagent test (Gutfinger, 1981). 0.5 mL of appropriately diluted samples or standard solutions of gallic acid were pipetted into 5 mL of distilled water, 0.5 mL of Folin-Ciocalteu reagent, and the mixture was allowed to react for 3 min. 1 mL of 20% Na_2CO_3 solution was added and mixed well, then left to stand for 1 h at room temperature for colour development. Absorbance was measured at 725 nm and the total phenolic content was derived by comparison with a gallic acid standard curve. The estimation of total phenolics in all the extracts was carried out in triplicate using a Shimadzu 1240 MINI UV-VIS spectrophotometer.

Phenolic profile determination of Clementine peel extracts

The methanol extract was taken to dryness in vacuo and the residue was partitioned between water and butanol. Chromatographic separation and identification of phenolic compounds in the extracts were performed on an LC-DAD-MS (ESI+) setup consisting of 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 x 2 mm, 4

µ, Superspher 100-4 (RP-18) column (Macherey–Nagel) kept at 40°C, at a flow rate of 0.33 mL/min, and at an injection volume of 5 µL (the sample solutions had an average concentration of = 10-20 mg extract/mL of methanol). For the gradient elution, the following program was used: (A) H_2O (containing 2.5% AcOH); (B) MeOH:H_2O (2.5% AcOH) (6:4), isocratic at 95% A for 2 min, then 0% A in 20 min, followed by 10 min isocratic wash at 0% A. The data were processed with the Xcalibur 1.2 software. The analysis was monitored at 278 and 340 nm and by ESI in the positive mode at a probe temperature of 450°C, probe voltage of 4.9 kV and at 20 and 60 eV in the mass analyzer.

DPPH radical scavenging activity

The free radical scavenging activity was determined by the reduction reaction between DPPH solution and sample extracts (Milardović *et al*., 2006). 100 µL of various concentrations of the extracts was added to 2.9 mL of DPPH solution (6 x 10^{-5} M). The mixture was shaken, allowed to stand at room temperature in the dark and the decrease in absorbance at 515 nm was measured after 30 min. All tests were performed in triplicate. The absorbance of blank, which is made from 2.9 mL of DPPH and 100 µL of methanol, was measured at t = 0. The scavenging of DPPH was calculated according to the following equation:

DPPH scavenging activity (%) = [(Abs (t = 0) - Abs (t = 30) / Abs (t = 0)] x100;
Where Abs (t = 0) = absorbance of DPPH radical + methanol at t = 0 min;
Abs (t=30) = absorbance of DPPH radical + phenolic extracts at t = 30 min.

Reducing Power

The reducing power was determined according to the method of Oyaizu (1986). Each extract in methanol (2.5 mL) was mixed with 2.5 mL of 200 mM sodium phosphate buffer (pH 6.6, and 2.5 mL of 1% potassium ferricyanide, and the mixture was incubated at 50°C for 20 min in water bath. After incubation, 2.5 mL of 10% trichloroacetic acid (w/v) were added; the mixture was centrifuged at 6000 rpm for 10 min. The upper layer (2.5 mL) was mixed with 2.5 mL of distilled water and 0.5 mL of 0.1% ferric chloride, and the absorbance was measured at 700 nm against a blank.

Statistical analysis

The results are expressed as means ± SD. All measurements were replicated three times. In order to study the influence of cultivar on the chemical composition of fruits, data were processed for analysis of variance (ANOVA), and the least significant difference (LSD) test was performed to compare the means ($P < 0.05$).

Results and discussion

Mineral contents of Clementine and Mandarin peels

The mineral contents (mg/g) of the Clementine peels on a dry weight basis are presented in Table 1. All the cultivars under investigation demonstrated varying amounts of minerals. The overall results revealed that K (5.39–7.45) (mg/g), Ca (2.42–2.76), Na (1.96–2.77), Fe (1.50–2.21), and Mg (0.84–1.92) were the major minerals followed by Zn (0.10–0.15), Mn (0.02–0.06) and Cu (0.01–0.04). Since some fruits are characterized by a high content of potassium (Julian-Loaeza *et al*., 2011; Barros *et al*., 2012; Boudries *et al.*, 2012), potassium was indeed the mineral with the highest concentration in all cultivar peels and the maximum concentration was found in Cheylard cv. with 7,45 mg/ g dw, Followed by calcium which was the second most abundant mineral. The Mandarin and the Merme cv. presented the highest amounts of this mineral with 2.76 and 2.73 mg/g dw respectively, while sodium was most abundant in Cheylard cv. (2.77 mg/g dw).

Table 1. Mineral contents of Clementine and Mandarin peels (mg/g of dw)

Cultivars	Na	K	Mg	Ca	Fe	Mn	Cu	Zn
Mandarin	2.19± 0.02^{c}	5.39± 0.03^{g}	0.95± 0.01^{d}	2.76± 0.03^{a}	2.06± 0.01^{b}	0.06± 0.00^{a}	0.02± 0.00^{b}	0.15± 0.00^{a}
Rocamora	2.11± 0.01^{d}	6.91± 0.01^{b}	0.96± 0.01^{d}	2.64± 0.04^{ab}	2.18± 0.01^{a}	0.05± 0.00^{b}	0.01± 0.00^{c}	0.13± 0.00^{c}
Merme	2.24± 0.04^{c}	5.68± 0.01^{d}	1.92± 0.01^{a}	2.73± 0.04^{a}	2.20± 0.01^{a}	0.04± 0.00^{c}	0.01± 0.00^{c}	0.10± 0.00^{e}
Cheylard	2.77± 0.02^{a}	7.45± 0.01^{a}	0.84± 0.01^{e}	2.47± 0.06^{b}	1.57± 0.01^{d}	0.03± 0.00^{d}	0.01± 0.00^{c}	0.14± 0.00^{b}
St Martin	2.68± 0.06^{b}	5.64± 0.02^{e}	1.11± 0.01^{c}	2.42± 0.05^{b}	1.83± 0.01^{c}	0.03± 0.00^{d}	0.01± 0.00^{c}	0.11± 0.00^{d}
Cadoux	2.07± 0.06^{d}	5.45± 0.01^{f}	1.11± 0.01^{c}	2.43± 0.06^{b}	1.50± 0.01^{e}	0.02± 0.00^{e}	0.02± 0.00^{b}	0.14± 0.00^{b}
Monreal	1.96± 0.03^{e}	6.83± 0.02^{c}	1.39± 0.02^{b}	2.63± 0.33^{ab}	2.21± 0.04^{a}	0.06± 0.00^{a}	0.04± 0.00^{a}	0.11± 0.00^{d}

Mean values of the same column, followed by the same letter, are not statistically different ($p<0.05$).

In addition to the health benefits of minerals, many antioxidant defenses depend on micronutrients. Some minerals are components of antioxidants enzymes: superoxide dismutase depends on Mn, Cu and Zn; catalase depends on Fe, and glutathione peroxidase on Se (Evans and Halliwell, 2001). The concentrations of iron were different among cultivars. Monreal, Merme and Rocamora cultivars had the highest concentrations, which showed 2.21, 2.20 and

Table 2. Means of total phenol, flavonoid and carotenoid contents per 100g dw of Clementine and Mandarin peels

Fruit	Total Phenols (mg gallic acid)	Flavonoids (mg catechin)	Carotenoids (mg β –carotene)
Mandarin	9686.2 ± 143.8^{e}	824.7 ± 49.6^{c}	75.3 ± 1.3^{ab}
Rocamora	10493.3 ± 292.6^{d}	784.1 ± 72.2^{cd}	76.0 ± 0.7^{a}
Merme	10730.3 ± 181.0^{cd}	942.5 ± 86.8^{ab}	58.1 ± 2.6^{d}
Cheylard	9773.0 ± 58.3^{e}	789.4 ± 36.3^{cd}	51.9 ± 1.1^{e}
St Martin	11079.5 ± 269.6^{bc}	701.8 ± 68.2^{d}	70.0 ± 1.8^{c}
Cadoux	11934.5 ± 312.3^{a}	1047.2 ± 54.2^{a}	72.9 ± 2.1^{bc}
Monreal	11281.1 ± 161.0^{b}	851.6 ± 57.5^{bc}	57.1 ± 1.2^{d}

Mean values of the same column, followed by the same letter, are not statistically different ($p<0.05$)

2.18 mg/g dw, respectively. While the highest value of Mg content was found in Merme cv. (1.92 mg/g dw). With regards to trace minerals (Zn, Mn, Cu), all the cultivars presented a content within the range of 0.01– 0.15 mg/ g dw.

According to Turra *et al*. (2011), there is a variable distribution of chemical elements in the diverse parts (seed, pulp, and peel) of sweet oranges, and in general the peel is richer than the pulp. The average concentrations of chemical elements determined in the peel of sweet oranges decreased in the following order K> Ca> Fe> Na> Zn. While, Özcan (2012) found that the major mineral contents of orange peel were established as Ca, K, P, Mg, Na, and among the minor metals determined Fe, Zn, Cu and Mn were found with different values than those obtained by Turra *et al*. (2011) and us.

The concentrations that we obtained were sometimes lower (Ca and K), close (Mg and Cu) or higher (Fe, Na, Mn and Zn) to the concentrations published by Özcan *et al.* (2012) in the orange peel. Many factors could influence the mineral and trace element concentrations; they include the species or cultivar and the specific plant organ (genetic factors), sampling period, its maturity and soil conditions. (Julian-Loaeza *et al*., 2011; Boudries *et al.*, 2012; Özcan *et al*., 2012).

Bioactive compounds of Clementine and Mandarin peels

The total phenolic, flavonoid and carotenoid contents of the Clementine cultivar peels are presented in Table2. The studied Clementine cultivars were grown in the same field sharing the same conditions, so the difference of bioactive compound contents, among Clementine peels, is mainly due to the cultivar factor.

Determination of carotenoids

Citrus fruits are complex sources of carotenoids, containing the largest number of carotenoids found in any fruits, these natural pigments are responsible for the external and internal colour of Citrus fruits and at the same time they have a significant impact on the commercial and nutritional quality of the fruit. The total carotenoid content was much lower than that of total polyphenols and flavonoids, and ranged from 51.9 ± 1.1 to 76.0 ± 0.7 mg/100 g dw (β-carotene equivalents). Significant differences in the contents of carotenoids were also observed in selected citrus species (Table 2).

The peel extract of *C. Clementina* Rocamora cv. contained the highest level of carotenoids with 76.0 ± 0.7 mg/100 g dw followed by *C. Mandarin* with a content of 75.3 ± 1.3 mg/100 g dw. *C. Clementina* Monreal cv. and Cheylard cv. showed the lowest value (57.1 ± 1.2 and 51.9 ± 1.1, respectively). Guimarães *et al*. (2010) found that the orange peel contains 31.57 µg/g extract which is lower than what we have found; this difference could be due to specie or other uncontrollable factors such as maturation, sunshine period, rainfall, temperature and geographical origin (Boudries *et al.*, 2007).

Total flavonoid contents

With regard to the flavonoid content (table 2), the levels ranged from 701.8 ± 68.2 to 1047.2 ± 54.2 in related cultivars. The highest amounts of flavonoids were assayed in *C. Clementina* Cadoux cv. (1047.2 ± 54.2 mg/100g dw Catechin equivalents) followed by Merme cv. (942.5 ± 86.8). The lowest amounts were observed in Rocamora cv. and St Martin cv. (784.1 ± 72.2 and 701.9 ± 68.2 mg/100g dw Catechin equivalents, respectively). Significant differences ($p<0.05$) were observed between flavonoid levels of peels of different cultivars.

The flavonoid contents of the citrus peel extracts are unique to each citrus fruit (Choi *et al*., 2007), and according to Nogata *et al.* (2006), the abundance order of flavonoid compounds in Clementine peel are hesperidin, narirutin, diosmin, isorhoifolin, neoponcirin, tangeretin, nobiletin, heptamethoxyflavone, eriocitrin, sinensetin, rutin. A content of 5.7 mg quercetin equivalent/g of extract powder in Clementine peel has been reported by Ghasemi *et al.* (2009), furthermore Goulas and Manganaris (2012) found that the *C. Sinensis* Valencia cultivar has 2.28 (mg rutin/ g dw) which is

Table 3. Peak assignments for analysis of Cadoux cv. peel extracts.

Rt (min)	$[M+H]^+$ (*m/z*)	$[M+Na]^+$ (*m/z*)	$[A+H]^+$ (*m/z*)	Other ions (*m/z*)	UVλmax (nm)	Identification
13.17	—	349	—	163	238, 309	Caffeic acid glucoside
15.01	195	—		177, 379	244, 320	Ferulic acid glucoside
15.74	—	409	—	—	242, 330	Unknown
16.85	—	409	—	—	244, 330	Unknown
18.42	595	617	—	—	238, 270, 340	6,8-di-C-glucopyrano sylapigenin
19.80	—	—	—	489, 565	240, 274, 340	Unknown
20.28	449	—	303	—	256, 266, 356	Quercetin rhamnoside (Quercitrin)
23.12	595	617	287	—	254, 266, 344	Luteolin rutinoside
23.63	611	633	303	—	255, 355	Rutin
24.28	611	633	303	177, 185	238, 284, 328	Hesperidin
26.99	—	—	—	509, 531	252, 270, 346	Unknown
29.52	772	—	303	163, 309	240, 286, 318	Ester of caffeic acid with hesperidin
30.24	637	—	329	—	240, 270, 342	Trimethoxyflavone rutinoside
34.13	725	747	429	—	254, 344	Natsudaidain derivative
36.70	373	395	—	435	240, 264, 332	Pentamethoxyflavone
37.31	403	425	—	485	248, 338	Nobiletin
39.11	403	425	—	485	248, 336	Hexamethoxyflavone
39.67	433	455	—	515	254, 344	Heptamethoxyflavone
41.42	373	395	—	—	238, 270, 328	Sinensetin

higher than the corresponding pulp and lower than our results

The presence and/or concentrations of flavonoids can be affected by the species, varieties and fruit development stages (Choi *et al*., 2007; Boudries *et al*., 2012). In fact, Choi *et al*. (2007) noticed that, in general, the total amount of flavonoids within the different mature citrus fruit peels was significantly lower than those of the immature fruit peels, confirming that the flavonoid contents in citrus fruit peels change dramatically during maturation. Furthermore, Vanamala *et al.* (2006) reported that the naringin content of grapefruit juice from the same grove and trees fluctuates during a season and varies considerably between crop years.

Total phenolic contents

The extraction of polyphenols from fruit peels is a crucial step for the phytochemical analysis and in vitro evaluation of antioxidant properties. Methanol is used to extract flavonoids aglycones and glucosides, limonoids, ascorbic acid, sugars and citric acid. However, sugars and organic acids may interfere in the colorimetric tests for phytochemical study, such as the Folin–Ciocalteu assay.

The total polyphenol contents ranged from 9686.2 ± 143.8 to 11934.5 ± 312.3 mg gallic acid equivalents/ 100g dw peels. The differences among the Clementine cultivars were monitored, and the contents of total phenolics exhibited the following order: Cadoux> Monreal> St Martin> Merme> Rocamora> Cheylard. Cadoux cv. had the maximum amount with 11934.5 ± 312.3 mg GAE/100g dw while Cheylard peel had the lowest level. Total phenolic contents are always substantially higher in the peel than in the pulp for different Citrus fruits, (Ghasemi *et al*., 2009; Goulas and Manganaris, 2012), which is in accordance with our results compared to the corresponding Clementine cultivar pulps previously studied (Boudries *et al*., 2012).

According to Ghasemi *et al.* (2009), the total phenolic contents ranged from 104.2 to 223.2 mg gallic acid equivalent/g extract powder of 13 different citrus fruits, and the Clementine peel phenolic content was 161.7 mg gallic acid equivalents/g of extract powder, which is higher than our results. Whereas Guimarães *et al.* (2010) found that the phenolic content in orange peel was 79.75 (mg GAE/g extract), which is lower than our result. As far as we know, there has been no such investigation on these Clementine cultivars. The total phenolic contents of citrus peels were affected by the method of peel preparation, the type of the solvent and its concentration, the operating temperature and the type of citrus peel used in the extraction, the, extraction time, (Li, Smith *et al*., 2006; Boudries *et al*., 2012). Furthermore, Ye *et al*. (2011) found that total phenolic contents were significantly different in the various mandarin cultivars.

LC-DAD-ESI/MS Analysis

Table 3 shows retention times, mass spectrometry ions and UV max of the identified compounds in Cadoux cv. peel. Since Citrus fruits contain a wide range of flavonoids (Nogata *et al*., 2006), their diversity was further examined in Cadoux cv. by means of LC-DAD-ESI/MS. Identification of the flavonoids

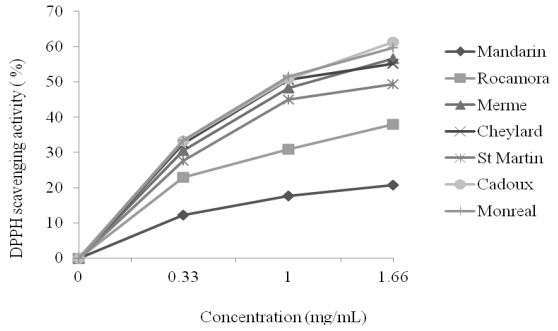

Figure 1. Effect of extract concentrations of Clementine and Mandarin peels on DPPH scavenging percentage
Mean values of the same column, followed by the same letter, are not statistically different ($p<0.05$)

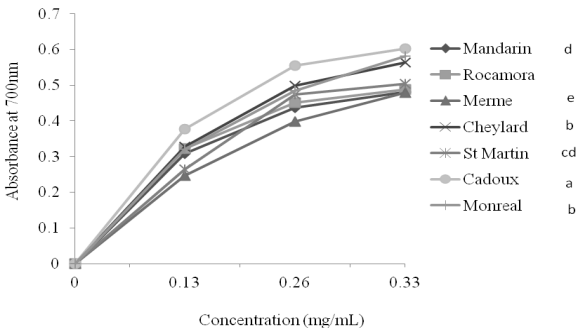

Figure 2. Effect of extract concentrations of Clementine and Mandarin peels on reducing power
Mean values of the same column, followed by the same letter, are not statistically different ($p<0.05$).

was based on UV spectra and on the characteristic fragmentation pattern in MS and the comparison with published MS data. Two wavelengths were used to monitor the elution: 278 nm for the determination of flavanone glycosides and 340 nm for flavone and flavonol glycosides.

Fifteen peaks were identified, and four were unknown, and the chromatogram indicated that hesperidin was the major flavonoid in the peel of Clementine peel, followed by Heptamethoxyflavone and hexamethoxyflavone. The characterisation of other compounds, found at lower concentrations, was also carried out and the total ion chromatograms revealed the presence of phenolic acid glucosides, flavanone glucosides and polymethoxylated flavones in the peel of Clementine fruit. The presence of glucoside derivatives and polymethoxyflavones demonstrated that the extraction of phytochemicals was efficient and no degradation of extracted polyphenols was monitored.

Peaks 1–4 were attributed to glucosides of phenolic acids as their UV/Vis spectra have a peak between 305 and 330 nm, which is indicative for hydroxycinnamic acids and their derivatives. The identification of different flavone glycosides and flavanones (5–12) was confirmed by LC–MS data. Polymethoxyflavones (compounds 13–19) displayed typical UV spectra of flavonoids, consisted of two absorption bands at 260–280 nm and 330–350 nm), these compounds were found at high concentrations in the peel and their peaks revealed that they were, together with hesperidin, the major group in Clementine peel. The presence of most of the compounds, such as hesperidin, sinensetin, rutin and nobiletin among others, has been reported in many Citrus fruits, such as Clementine, Mandarin, sweet and bitter oranges (Li *et al*., 2006; Nogata *et al*., 2006; Abad-Garcia *et al*., 2012) whereas the presence of natsudaidain derivative is reported for the first time here in Clementine peel.

Antioxidant activity

Previous studies demonstrated that it is not appropriate to use one-dimensional methods to evaluate the antioxidant activity of multifunctional food such as fruits and vegetables, since they contain a large diversity of natural antioxidants. Therefore, an approach with multiple assays for determination of antioxidant activity is highly advisable. The antioxidant activity of Citrus extracts, determined with two different assays, showed a similar trend in all protocols.

DPPH radical scavenging activity

The 2, 2-diphenyl-1-picrylhydrazyl radical (DPPH.) is a stable organic nitrogen radical, is commercially available and has a deep purple color. The radical scavenging activity (RSA) assay measures the reducing capacity of antioxidants toward DPPH.. Upon reduction, the colour of DPPH. solution fades and this color change is conveniently monitored spectrophotometrically at 517 nm. Therefore, test compounds with high antioxidant activity result in a rapid decline in the absorbance of the DPPH. and a lower absorbance indicates a higher scavenging effect (Guimarães *et al*., 2010).

When a solution of DPPH. is mixed with a substance that can donate a hydrogen atom, the reduced form of the radical is generated accompanied by loss of colour. Representing the DPPH. by X. and the donor molecule by AH, the primary reaction is:

$$X. + AH \longrightarrow XH + A.$$

All the citrus fruit extracts exhibited a concentration dependent DPPH radical scavenging activity (Figure 1).

The Cadoux cv. And Monreal cv. were shown to be the most active material, followed by Cheylard cv. and Merme cv., while Mandarin clearly showed a lower antioxidant capacity.

Reducing power

In this assay, the yellow colour of the test solution changes to various shades of green and blue depending on the reductive agent content. The presence of reductants such as antioxidant substances in the samples causes the reduction of the Fe^{3+}/ ferricyanide complex to the ferrous form. Therefore, Fe^{2+} can be monitored by measuring the formation of Perl's Prussian blue at 700 nm. Figure 2 shows the reducing power of the different cultivar methanolic extracts as a function of their concentrations. The reducing ability of extracts augmented with increasing concentration of samples in the medium.

The values of absorbance at 700 nm for the peel extracts of all Clementine and Mandarin cultivars revealed that all samples had a capacity to reduce iron (III) and had electron donor properties for neutralizing free radicals The Reducing Power values of Clementine cultivars and Mandarin were different, these differences were statistically significant ($p<0.05$) and decreased in the order of Cadoux, (Monreal, Cheylard), Rocamora, St Martin, Mandarin and Merme.

A plethora of data from previous studies indicated that the phytochemical profile and antiradical scavenging activity may significantly vary among Citrus species and cultivars (Guimarães *et al*., 2010; Ramful *et al*., 2011; Ye *et al*., 2011; Boudries *et al*., 2012; Goulas and Manganaris, 2012; Moulehi *et al*., 2012). Furthermore, Halvorsen *et al*. (2002) reported that geographic origins influenced the antioxidant activity in fruit samples collected from different regions in the world.

Antioxidant activity of fruits and vegetables significantly increases with the presence of high concentration of total polyphenol content. In the present study, the correlation between total phenolic and flavonoid contents with radical scavenging activity and reducing power of methanolic extracts from clementine and mandarin peels were analyzed, and linear regression plots were generated and their relationship was investigated with a view to rationalize the antioxidant properties of extracts in terms of their bioactive constituents.

The correlation between total phenol contents and antioxidant activity has been widely studied in different citrus fruits (Ghasemi *et al*., 2009). However, there are contrasting reports whereby phenolic compounds make significant contributions to the antioxidant activity of citrus. Some previous works demonstrated a high correlation coefficient of the total phenolics and antioxidant activity for Citrus extracts (Ramful *et al*., 2011). Regarding our results, the extracts with high phenolic content presented potent antioxidant capacity, with significant correlation for the DPPH test (R=0.60). However, without presenting a direct correlation (R=0.26) for reducing power, which is in agreement with studies in other Citrus fruits (Anagnostopoulou et al., 2006; Ghasemi *et al*., 2009; Goulas and Manganaris, 2012).

The lack of correlation observed between the antioxidant activity and the phenolic content of the extracts, shows that other substances must be responsible for the major part of the efficiency of the extracts such as non-phenolic compounds like vitamin C (Apak *et al*., 2007). Regarding flavonoids, there was a poor correlation between total flavonoids and antioxidant activity too, which is in agreement with the literature (Anagnostopoulou *et al*., 2006; Ghasemi *et al*., 2009). It is known that only flavonoids with a certain structure and particularly the number and the position of hydroxyl group in the molecule can act as proton donating and show antioxidant activity (Rice-Evans *et al*., 1996). Furthermore, the extracts are very complex mixtures of many different compounds with distinct activities.

It is suggested that the antioxidant activity of mixture of polyphenols is not likely to be ascribed to the property of an individual compound but rather to the synergistic actions of several phytochemicals. In fact, Freeman *et al.* (2010) found that synergistic interactions between phenolic compounds at the concentration found in navel oranges was found to be true. The interaction between naringenin and hesperidin provided the most synergism, while the addition of a 3rd compound enhanced that synergism. Otherwise, the use of the whole extract instead of individual antioxidants allows taking advantage of additive and synergistic effects of different phenolics, flavonoids, ascorbic acid, carotenoids and reducing sugars present in the samples.

Conclusion

To the best of our knowledge, this is the first report indicating the phytochemical profile of Clementine cultivar peels, grown in Algeria. Interestingly, results showed that these peels contain a plethora of polyphenolics with a high antioxidant activity that can potentially be useful in the prevention of diseases in which free radicals are involved. This study provides also additional data on mineral content that Clementine peels contain and it turn out to be a promising source of several essential minerals like K, Ca, Na and Fe.

The results provide useful information for the eventual industrial exploitation of Clementine peels as agrifood waste of the Algerian citrus industry

within the concept of waste prevention, minimisation and valorisation as desirable solutions for waste management and in view of producing added value products for use in the pharmaceutical, food and cosmetics industries.

Finally, and in order to pinpoint radical scavenger phytochemicals, further studies on the evaluation of antioxidant capacity of individual compounds the existence of possible synergisms among the compounds with the employment of sophisticated assays is needed; furthermore, the mechanisms by which they protect against disease development stands a challenging perspective highly warranted.

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