Revue Agrobiologia
www.agrobiologia.net
ISSN (Print): 2170-1652
e-ISSN (Online): 2507-7627

CHARACTERIZATION OF THE PHENOLIC COMPOSITION AND ASSESSMENT OF ANTIOXIDANT ACTIVITY OF PEELS FROM ORANGES GROWN IN ALGERIA

LAGHA-BENAMROUCHE Samira*

Research Laboratory Soft Technology, Valorization, Physiochemistry of Biological Materials and Biodiversité, Faculty of Sciences, UMBB University, Boumerdes 35000, Algeria

Reçu le 05/12/2021, Révisé le 18/04/2022, Accepté le 25/04/2022

Abstract

Topic Description: The citrus fruit consumption and processing industry consumed gigantic masses of by-products such as peels. These are a natural source of bioactive compounds. Few studies have been carried out so far on orange tree by-products, in particular on the peel of some blond and pigmented varieties. The targeted themes particularly concern the analysis of the chemical composition, the identification of the lipid fraction and the study of the antimicrobial and antioxidant activities of certain varieties of oranges (Navels and bitter).
Objective: The objective of this study is to determine and compare the antioxidant activity of the peels of seven varieties of oranges cultivated in Algeria and to characterize the phenolic composition by HPLC-DAD analysis.
Method: Profiles of free phenolic acids and flavonoids were analyzed by HPLC-DAD, while antioxidant capacities were assessed in vitro using scavenging assay of hydrogen peroxide (HPS), phosphomolybdate method (PMM) and ferrous ion chelating ability (FIC).
Results: Among the cultivars tested, Bigarade and Double fine had the strongest antioxidant capacities. Two phenolic acids and seven flavonoids were identified and quantified. Kaempferol was the major flavonoids of *C. sinensis* L. However, hesperidin, poncirin, apigenin-7-glycoside, naringin, narirutin, and rutin made up the majority of the total flavonoids in the peels of *C. aurantium* L.
Conclusion: orange peels are a natural and renewable source of antioxidants
Key words: Antioxidants, identification, orange, peels, phenolic compounds.

CARACTÉRISATION DE LA COMPOSITION PHÉNOLIQUE ET EVALUATION DE L'ACTIVITÉ ANTIOXYDANTE DES ECORCES D'ORANGES CULTIVÉES EN ALGÉRIE

Résumé

Description du sujet : La consommation et l'industrie de transformation des agrumes génèrent de gigantesques masses de sous-produits tels que les écorces. Ces derniers sont une source naturelle de composés bioactifs. Peu d'études ont été réalisées, jusqu'à présent, sur les sous produits d'orangers notamment sur les écorces de certaines variétés blondes et pigmentées. Les thématiques traitées concernent particulièrement l'analyse de la composition chimique, l'identification de la fraction lipidique et l'étude des activités antimicrobienne et anti-oxydante de certaines variétés d'oranges (Navels et amères).
Objectif : L'objectif de cette étude est de déterminer et de comparer l'activité antioxydante des écorces de sept variétés d'oranges cultivées en Algérie et de caractériser la composition phénoliques par analyse HPLC-DAD.
Méthodologie : Les profils des acides phénoliques libres et de flavonoïdes ont été analysés par HPLC-DAD, tandis que les capacités antioxydantes ont été évaluées *in vitro* en utilisant un test de piégeage du peroxyde d'hydrogène, la méthode au phosphomolybdate et la capacité de chélation des ions ferreux.
Résultats : Parmi les cultivars, Bigarade et Double fine possédaient les plus fortes capacités antioxydantes. Deux acides phénoliques et sept flavonoïdes ont été identifiés et quantifiés. Le kaempférol était le principal flavonoïde de *C. sinensis* L. Cependant, l'hespéridine, la poncirine, l'apigénine-7-glycoside, la naringine, la narirutine et la rutine constituaient la plus grande partie des flavonoïdes totaux dans les écorces de *C. aurantium* L.
Conclusion: orange peels are a natural and renewable source of antioxidants
Mots clés : Antioxydants, composés phénoliques, écorces, identification, orange.

* Auteur correspondant : LAGHA-BENAMROUCHE Samira. Email : s.lagha@univ-boumerdes.dz

INTRODUCTION

Citrus is a common term type of flowering plants in the family *Rutaceae.* Approximately 34% of *Citrus* is processed into juice; therefore, a large amount of wastes including peels, pulps, rags and seeds are formed each year [1]. During the processing of *Citrus* juices; peels are the primary by product. If it's not processed, the peels become waste and possible source of environmental pollution. In fact, the peels, in particular, are an abundant source of phytochemicals (e.g., carotenoids, pectin, flavonoids) which contribute to health [2].

Few studies have been carried out so far on the by-products of orange trees, especially on the peels of certain blond and pigmented varieties. The themes dealt with concern in particular the analysis of the chemical composition, the identification of the lipid fraction and the study of the antimicrobial and antioxidant activities of certain varieties of oranges (Navels and bitters) [3, 4]. Unfortunately, in Algeria no study has been undertaken. Our interest in these products comes from these observations and the present work consists in filling the lack of information on the properties of the orange peels of local origin. The objective of this research is to determine and compare the antioxidant activity of peels of seven varieties of orange (Washington Navel, Thomson Navel, Sanguinelli, Double Fine, Portuguese, Jaffa and Bitter) grown in Algeria and characterize the composition of phenolic compounds by HPLC-DAD analysis.

MATERIAL AND METHODS

1. Preparation of sample

The fruits of seven varieties of oranges were harvested at optimum maturity from the region of Bejaia (Timezrit and Amizour) (North East of Algeria). The cultivars were: sweet orange (Thomson, Washington, Sanguinelli, Double Fine, Portugaise and Jaffa) and sour orange (Bigarade**)**. The index of maturity of the juices of oranges tested (sugar/acidity ratio (g/l)) was superior at 7, except for Bigarade. All the fruits were of eating quality and without blemishes or damage.

2. Extraction and HPLC analysis of phenolic compounds

2.1. Extraction and purification of phenolic compounds

3g of dried powder were extracted with 30 ml of methanol-water (80%) at room temperature for 22 hours using magnetic blender. Then, the extract was vacuum filtered through sintered glass filter crucibles (porosity 3) and the residue was taken up again with 30 ml of acetone-water (70%). The volumes of the two obtained filtrates were mixed and then centrifuged at 3060 g for 5 minutes and vacuum filtered using Whatman No. 1 paper. The obtained aqueous organic extract was concentrated, under reduced pressure at 40°C using a rotary evaporator.

The aqueous extract was washed with oil ether to eliminate the pigments (chlorophylls and carotenoids) then washed with the ethyl acetate. After a strong agitation and a decantation, the phenolic compounds pass in the ethyl acetate and sugars remain in the aqueous phase. Each operation was repeated 4 times. The organic phase was recovered and concentrated in rotary evaporator at 40°C, until complete evaporation and then reconstituted in pure methanol.

2.2. Analysis of phenolic compounds

Analysis were performed in triplicate on a Agilent 1100 HPLC system (Agilent Technologies, Palo Alto CA-USA) operated by Windows NT based ChemStation software equipped with a diode array detector (DAD), binary pump, degasser and auto sampler. The column used was a Beckman Ultrasphere ODS (Roissy CDG, France): 4.6 mm×250 mm, 5 µm equipped with a precolumn 4.6 mm×10 mm (same granulometry). The mobile phase consisted of two solvents: Solvent A, water/formic acid (95/5; v/v) and Solvent B, acetonitrile/solvent A (60/40; v/v). Phenolic compounds were eluted under the following conditions: 1ml/min follow rate and the temperature was set at 25°C, isocratic conditions from 0 to 10 min with 0% B, gradient conditions from 0 % to 5 % B in 30 min, from 5 % to 15 % B in 18 min, from 15 % to 25 % B in 14 min, from 25 % to 50 % B in 31min, from 50% to 100% B in 3 min, followed by washing and reconditioning the column. The ultraviolet–vis spectra (scanning from200 to 600 nm) were recorded for all peaks. The identification of phenolic compounds were obtained by comparing the retention times and ultra-violet–visible spectra with authentic standards and with previously reported data in the literature [5]. The quantification of each identified compounds was performed on each samples using an external standard calibration curve for each compound.

The curves were obtained using the commercial standards of the concentrations normally present in extracts (approximately 1–100 mg.kg−1), obtaining regression coefficients (R^2) above 0.995 in all cases.

3. Antioxidant activity

3.1. Phosphomolybdenum method (PMM)

The total antioxidant capacities of the sample extracts were evaluated by the phosphomolybdenum method as described by Prieto et *al.* [6]. Quercetin and gallic acid were used for comparison at the concentration 0.2 mg/ml.

3.2. Hydrogen peroxide scavenging capacity (HPS)

Hydrogen peroxide scavenging ability of the peels extracts was performed according to the method of Ruch et *al.* [57] . Quercetin and gallic acid were used for comparison at the concentration 0.1 mg/ml. A percent inhibition versus concentration curve was plotted and the concentration of sample required for 50% inhibition was determined and expressed as IC_{50} value.

3.3. Ferrous ion chelating capacity (FIC)

The ferrous ion chelating capacity was determined as described by Bhandari and Kawabata [8]. EC_{50} value (mg extract/ml) is the effective concentration at which ferrous ions were chelated by 50% and was obtained by interpolation from linear regression analysis. EDTA and quercetin were used for comparison at the concentration 2.4 mg/ml and 2.4µg/ml, respectively.

4. Statistical analysis

The statistical analysis of the results was carried out using the STATISTICA 5.5 software and the degree of significance is taken at the probability $p \leq 0.05$. We performed a one factors analysis of variance followed by a Tukey's test. All data represent the mean of the three tests ± standard deviation.

RESULTS

1. Phenolic contents of the samples

The composition and concentrations of major phenolic substances that were determined by HPLC–DAD analysis are presented in table 1. The typical chromatogram recorded at 280 nm is presented in figure 1.Two free phenolic acids were identified in the peels orange cultivars, including gallic acid and ferulic acid. In the group of phenolic acids, gallic acid and ferulic acid was found in all cultivars. Gallic acid content varied significantly ($p \leq 0.05$) between cultivars, and ranged from 13.70 µg/g DM (Thomson) to 964.18 µg/g DM (Double fine). On the other hand, the proportion of ferulic acid varied significantly ($p \leq 0.05$) between cultivars, and ranged from 13.72 µg/g DM (Portugaise) to 277.44 µg/g DM (Double fine).

Table 1: Content of individual phenolic compounds of peels from selected orange varieties determined by HPLC-DAD (µg/g DM).

	Cultivars						
	Washington	Thomson	Sanguinelli	Double fine	Portugaise	Jaffa	Bigarade
Gallic acid	113.18± 0.86^{C}	13.70± 0.1^{F}	24.02±1.8^{D}	964.18±7.24^{A}	17.26±0.12^{E}	25.70±0.2^{D}	172.54±1.3^{B}
Ferulic acid	83.68±0.62^{E}	123.36±0.92^{C}	86.70±0.66^{D}	277.44±2.08^{A}	13.72±0.1^{G}	56.94±0.42^{F}	190.7±1.44^{B}
TPA	196.86	137.06	110.72	1241.62	30.98	82.64	363.34
Narirutin	63.58±0.48^{E}	173.12±01.3^{D}	209.24±1.58^{C}	273.70±2.06^{B}	2.34±0.02^{G}	17.66±0.14^{F}	565.80±4.24^{A}
Naringin	Nd	Nd	50.64±0.38^{B}	Nd	Nd	17.46±0.14^{C}	395.78±2.98^{A}
AP7G	57.28±0.44^{D}	55.48±0.42^{E}	133.52±1^{C}	168.22±1.26^{B}	2.04±0.02^{G}	8.80±0.06^{F}	351.82±2.64^{A}
Hesperidin	117.36±0.88^{E}	254.78±1.92^{B}	205.44±1.54^{C}	140.16±1.6^{D}	19.04±0.14^{G}	54.86±0.42^{F}	1442.32±10.84^{A}
Poncirin	Nd	27.44±0.20^{B}	8.34±0.06^{C}	Nd	5.56±0.04^{E}	6.98±0.06^{D}	77.56±0.58^{A}
Rutin	124.04±0.94^{D}	186.4±1.4^{B}	165.52±1.24^{C}	Nd	24.1±0.18^{F}	83.94±0.64^{E}	590.44±4.44^{A}
Kaempferol	2039.69±15.32^{A}	1596.68±12^{B}	1175.98±8.08^{D}	615.74±4.62^{F}	395.50±2.7^{G}	785.92±5.7^{E}	1281.34±9.62^{C}
TF	2400	2293.9	1849.08	1625.72	412.68	948.62	4705.06

AP7G: Apigenin7 glycosids. DM: Dry matter. Nd: Not determined. TF: Total flavonoids. TPA: Total phenolic acids. Each value in the table is the mean ± standard deviation (n = 3).Values in the same row sharing different letters are significantly different (p≤0.05). The results are sorted in decreasing order: A >B >C >D>E >F >G.

As shown in Table 1, the content of flavonoids identified were significantly different (*p≤0.05*) among the orange cultivars. Interestingly, analysis of the profiles of polyphenols by HPLC-DAD has seven quantization flavonoids that have not yet been quantified in Algeria orange by product.

These flavonoids are narirutin, naringin, hesperidin, poncirin, kaempferol, rutin and apigenin -7- glycoside (AP7G). The obtained results show that the flavones (AP7G) and the flavonols (kaempferol and rutin) and the flavanons glycosids (narirutin, hesperidin and rutin) neveals a ubiquitous distribution pattern compared to that of flavanons glycosids (naringin and poncirin). In the group of flavonoids, kaempferol was the major flavonoids in *C. sinensis* L. However, hesperidin, Poncirin, apigenin -7- glycoside, Naringin, Narirutin and rutin constituted constituted the greater part of total flavonoids in the peels *C. aurantium* L. Narirutin, AP7G, hesperidin and kaempferol were detected in all cultivars while some flavonoids were not found in all investigated cultivars in this study such as rutin, poncirin and naringin. Rutin.

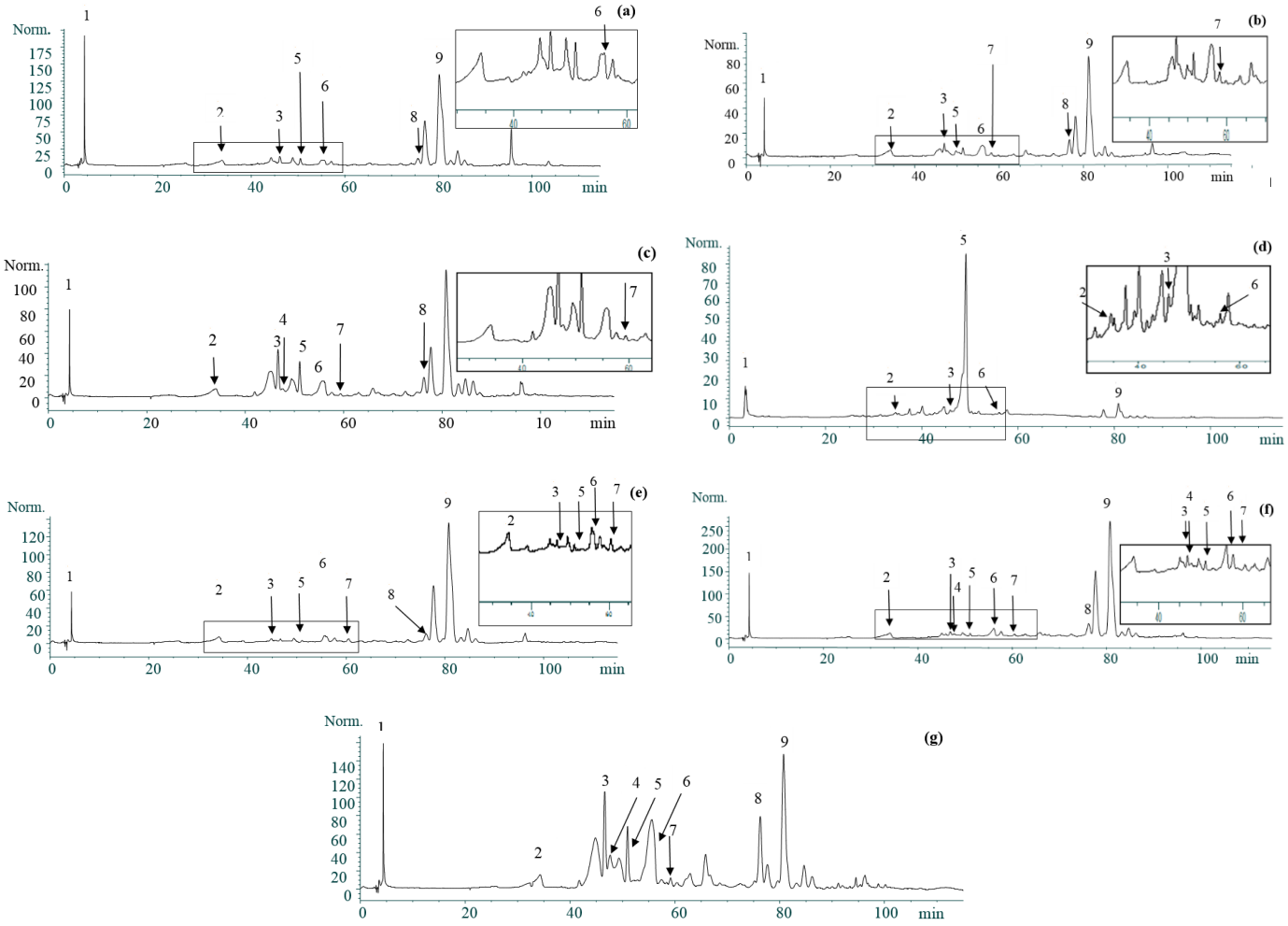

Figure 1: HPLC-DAD chromatograms of peels from selected orange varieties at 280 nm
(a) Washington, (b) Thomson, (c) Sanguinelli, (d) Double fine, (e) Portugaise (f) Jaffa (g) Bigarade . Peaks: 1-gallic acid, 2-ferrulic acid, 3-narirutine, 4-naringin, 5-Apigenin7 glycosids, 6-hesperidin, 7-poncirin, 8-rutin, 9-kaempferol.

2. Antioxidant activity

The analysis of the reducing power of peels at concentration of 1 mg/ml, with the phosphomolybdenum method resulted absorbances between 0.27 to 0.75. As can be seen in table 2, Bigarade variety presents the most pronounced reducing power but significantly lower ($p \leq 0.05$) than that of the gallic acid used as standard for quantification. A similarity of reducing power ($p > 0.05$) was observed between quercetin and peels of Bigarade. Concerning the ferrous ion chelating capacity of samples tested at the concentration of 12 mg/ ml, the results consigned in table 2 revealed clearly that significant differences ($p \leq 0.05$) in the FIC power were noticed between the cultivars. The FIC capacity ranged from 57.13 to 75.46 % for peels.

Thomson variety has the strongest FIC capacity (75.46 %), whereas Portugaise and Jaffa cultivars exerted the lowest values (57.13 and 60.28%, respectively). The hydrogen peroxide scavenging capacity values for the investigated extracts ranged from 52.90 to 77.42 %. Bigarade presents the highest inhibition activity (77.42%). The results of ANOVA analysis indicated that antioxidant activity of peels is significantly lower ($p \leq 0.05$) than that of gallic acid. The peels of Bigarade and Thomson prove more active than the quercetin.

Table 2: Antioxidant activities of peels from selected orange varieties and standards

Cultivars / standards	PMM (absorbance)	HPS (%)	FIC (%)
Washington	0.38±0.01^{F}	52.90±0.89^{G}	61.80±0.84^{G}
Thomson	0.56±0.02^{C}	72.47±0.86 ^{c}	75.46±1.12 ^{C}
Sanguinelli	0.49± 0.01^{D}	62.99±0.60 ^{F}	70.75±1.30^{DE}
Double fine	0.39±0.01^{F}	60.96±0.07 ^{F}	66.41±1.34 ^{F}
Portugaise	0.54±0.01^{C}	65.69±0.14^{E}	57.13±2.23 ^{I}
Jaffa	0.46±0.00^{E}	61.15±0.31^{F}	60.28±0.39 ^{GH}
Bigarade	0.75±0.01^{B}	77.42±0.16 ^{B}	73.20±2.23^{CD}
Gallic acid	0.97±0.01 ^{A}**	90.00±0.20 ^{A}*	-
Quercetin	0.76±0.00 ^{B}**	67.04±0.10 ^{D}*	81.89±0.34 ^{B} ***
EDTA	-	-	97.67±0.28 ^{A} ****

PMM: phosphomolybdate method. FIC: Ferrous ion chelating capacity. HPS: Hydrogen peroxide scavenging capacity. Each value in the table is the mean ± standard deviation (n = 3).Values for the same method sharing different letters are significantly different ($p \leq 0.05$). The results are sorted in decreasing order: A >B >C >D>E >F >G >H>I. * at 0.1 mg/ml. ** at 0.2 mg/ml. *** at 2.4 mg/ml.**** at 2.4µg/ml

3. Correlation

The calculated IC_{50} and EC_{50} are reported in the Table 3. Low IC_{50} corresponds to a strong inhibitory capacity of H_2O_2. The values indicate that the IC_{50} of quercetin is significantly lower ($p \leq 0.05$) followed by gallic acid. The highest IC_{50} is recorded for the peels of Washington, Sanguinelli, Double fine and Jaffa. EC_{50} corresponds to strong chelate metal ions. The data shows that the EC_{50} of the EDTA is significantly lower ($p \leq 0.05$) followed by the quercetin. The highest EC_{50} is recorded for the peels of Portugaise, Jaffa, Washington and Double fine.

Table 3: IC_{50} of the H_2O_2 Scavenging capacity and EC_{50} of Chelating effect of peels and standards

	IC50 (mg/ml)	EC50 (mg/ml)
	HPS (%)	FIC (%)
Washington	0.911±0.026^{G}	9.709±0.651 ^{EFG}
Thomson	0.671±0.022 ^{D}	7.955±0.775 ^{C}
Sanguinelli	0.806±0.021 ^{F}	8.451±0.575 ^{CD}
Double fine	0.833±0.037 ^{F}	8.955±0.605 ^{DE}
Portugaise	0.727±0.023 ^{E}	10.652±1.015 ^{GH}
Jaffa	0.817±0.012 ^{F}	10.038±0.220^{G}
Bigarade	0.637±0.052 ^{C}	8.082±0.134 ^{CD}
Gallic acid	0.074±0.005 ^{B}	-
Quercetin	0.056±0.003 ^{A}	1.475±0.015 ^{B}
EDTA	-	0.0013±0.0003 ^{A}

IC50: Concentration of sample required for 50% inhibition.EC 50: Concentration at which ferrous ions were chelated by 50%.FIC: Ferrous ion chelating capacity. HPS: Hydrogen peroxide scavenging capacity. Each value in the table is the mean ± standard deviation (n = 3).Values for the same method sharing different letters are significantly different ($p \leq 0.05$). The results are sorted in crescent order: A <B <C <D<E <F <G <H.

The correlation coefficients between the antioxidant capacities and the phenolic contents of the peels were also determined (Table 4). A very weak correlation existed for the FIC and the HPS assays (r=0.41) and between the FIC and PMM essays (r=0.25). On the other hand, a correlations observed between the HPS and the PMM assays were much stronger (r=0.86).

The results presented in this study showed a weak correlation between the flavonoid contents of peels and, the FIC and the PMM assays (0.47 and 0.42, respectively), reflecting the moderate contribution of flavonoids to the antioxidant capacities of orange peels. On the other hand, a very weak correlation existed between the HPS capacity and phenolic acids and flavonoid contents (0.007 to 0.36),

and between FIC and the PMM assays and the phenolic acids contents of peels (0.019 and 0.067, respectively), implying that the phenolic acids may not be the main components responsible for HPS ability and FIC of the tested extracts.

Table 4: Correlation matrix between phenolic contents and antioxidant activities

	PMM	FIC	HPS	PAC	FC
PMM	-				
FIC	0.25	-			
HPS	0.86*	0.41	-		
PAC	0.067	0.019	0.007	-	
FC	0.42	0.47	0.293	0.02	-

Abbreviations: PMM: Phosphomolybdate method. FIC: Ferrous ion chelating capacity. HPS: Hydrogen peroxide scavenging capacity.PAC: Phenolic acids content. FC: Flavonoids content. *: Significantly different ($p \leq 0.05$).

The spider diagram is used to better visualize this relationship (Fig. 2). The findings of this study indicate that the peels of Bigarade present the highest levels of total phenols and present the most pronounced antioxidant activities followed by the peels of Thomson and Portugaise. Therefore, Washington that has low total phenolic content has lower antioxidant activities than the other studied extracts and show the moderate contribution of flavonoids to the antioxidant capacities of orange peels contrary to phenolic acids.

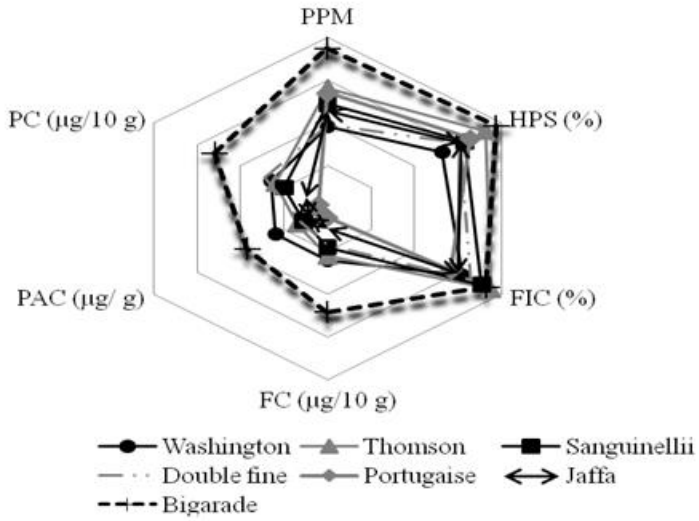

Figure 2: Comparison of antioxidant activity, total phenolic, phenolic acids and flavonoids contents of orange peels.

FC: flavonoids content. PAC: phenolic acids content. TPC: Total phenolic content. PMM: phosphomolybdate method. FIC: Ferrous ion chelating capacity. HPS: Hydrogen peroxide scavenging capacity.

DISCUSSION

In agreement with our discovery, Hayat et *al.* [9] and Choi et *al.* [10], also found gallic acid and ferulic acid as well as some hydroxybenzoic acid derivatives (i.e., protocatechuic, vanillic, syringic, salycilic and hydroxybenzoic acids) and some hydroxycinnamic acids (i.e., caffeic, *p*-coumaric, *m*-coumaric, cinnamic and sinapic acids) in *Citrus* peels (mandarin: *C. reticulate* Blanco cv. Kinow and chenpi: *C. unshiu* Kovich and *C. tachibana* Makino Tonaka and *C. reticulate* Blanco), respectively. Bocco et *al.* [3],

found that total content of phenolic acids (i.e., caffeic, *p*- coumaric, ferulic and sinapinic acids) in peels of sour orange (*C. aurantium* L.) was 2956 µg/g DM. On the other hand, Wang et *al.* [2] found that the total content of phenolic acids (i.e., caffeic, chlorogenic, ferulic, sinapic and *p*-coumaric acids) in sweet orange peels (*C. sinensis* L. osbeck) was 931.8 µg/g DM. Overall, these findings far exceed those obtained for our samples (sour and sweet oranges) (363.34 µg/g DM and 30.98 to 196.86 µg/g DM, respectively), except for the variety Double fine (1241.62 µg/g DM).

This can be explained by the amount of free phenolic acid compounds identified.

Regarding flavonoids, Wang et *al.* [2], have also identified rutin in peels of *C. sinensis* L. Wang et *al.* [2], Londono-Londono et *al.* [11] and Chen et *al.* [12], also reported the presence of hesperidin. This later, with narirutin and naringin have also been found in peels of sweet orange [13, 14]. Previous studies performed by Wang et *al.* [2], identified kaempferol in peels of sweet orange and that corroborates our results. Poncirin and AP7G identified in peels of sweet and sour oranges were not detected in other work on orange by product. Kim et *al.* [15], have found poncirin in peels of mandarin (*C. unshiu*) nevertheless, this flavanone was not quantified. According to Menichini et *al.* [16], apigenin is detected in the leaves of *C. medica* L. cv. Diamante (Diamante citron) but it is not detected in the peels. It is difficult to compare our results with historical data. Depending on the fruit development stage, the presence and/or concentrations of flavonoids may be affected. Most *Citrus* species accumulate substantial quantities of flavonoids during their tissue development [17]. Ortunõ et *al.* [18], found in a variety of grapefruit and pummelo, that the highest flavanone levels are detected during the juvenile stages of fruit development. On the other hand, Castillo et *al.* [19], demonstrated in *C. aurantium*, that the highest levels of pruning and hesperitin 7-*O*-glucoside are present during fruit development and falls sharply when the corresponding neohesperidosides, naringin and neohesperidin (the most abundant flavanone glycosides in *C. aurantium*) reach their maximum levels.

On the basis of the reducing power of peels, orange varieties are classified according to the following order: Bigarade > (Portugaise - Thomson) > Sanguinelli >Jaffa > (Double fine-Washington). The order of antioxidant capacity based on hydrogen peroxide scavenging capacity of peels was: Bigarade > Thomson > Portugaise > (Jaffa+Double fine+Sanguinelli) > Washington. According to the data, it can be seen that whatever the variety, the EDTA which proves the most FIC efficiency. Ferrous ion chelating activity of the peels is statistically inferior ($p \leq 0.05$) than that of the quercetin. Ferrous ion, commonly found in food systems, is well known as an effective pro-oxidant [20]. Polyphenols can chelate pro-oxidant metal ions, such as iron and copper, thus preventing free radical formation from these pro-oxidants [21]. Flavonoids were known to retain free radical scavenging capacity by forming complexes with metal ions [22]. The results presented in this study showed a weak correlation between the contents of flavonoids and the FIC and PMM assays. On the other hand, a very weak correlation existed between the levels of phenolic acids and the antioxidant activities tested, implying that the phenolic acids may not be the main components responsible for HPS ability and FIC of the tested extracts. These results corroborate with those of Zilic et *al.* [23], who reported a negative correlation between some phenolic acids such as ferulic acid and antioxidant capacity in durum wheat, determined by DPPH radical scavenging.

CONCLUSION

In this work we have proposed the characterization of the distinctive phenolic compounds from the peels of sweet and sour orange varieties. The antioxidant activity of the phenolic extracts peels from the same varieties was assessed using three methods (PMM, HSP and FIC). Two phenolic acids (gallic and ferulic acids), one flavones (AP7G), two flavonols (kaempferol and rutin) and four flavanons glycosids (narirutin, naringin, hesperidin and poncirin) were characterized in the first time by HPLC-DAD. Gallic acid and ferulic acid was found in all cultivars. Gallic acid content varied significantly ($p \leq 0.05$) between cultivars, and ranged from 13.70 µg/g DM (Thomson) to 964.18 µg/g DM (Double fine). On the other hand, the proportion of ferulic acid varied significantly ($p \leq 0.05$) between cultivars, and ranged from 13.72 µg/g DM (Portugaise) to 277.44 µg/g DM (Double fine). The quantification of the identified flavonoids has shown that the kaempferol was the major flavonoids in *C. sinensis* L. In contrast, hesperidin, Poncirin, apigenin -7- glycoside, Naringin, Narirutin and rutin constituted the greater part of total flavonoids in the peels of *C. aurantium* L. The data indicate also that Bigarade and Thomson varieties showed strong antioxidant activities: PMM absorbance (0.56-0.75), HPS (72.47-77.42 %) and FIC (73.20-75.46 %) when compared with other tested varieties (Washington Navel, Sanguinelli, Double fine, Portugaise, Jaffa), a fact which encourages the prospect of its recovery as a source of powerful natural antioxidants.

ABREVIATION

AP7G: Apigenin7 glycosids, DM: Dry matter, EC 50: Concentration at which ferrous ions were chelated by 50%, FC: flavonoids content, FIC: Ferrous ion chelating capacity, IC50: Concentration of sample required for 50% inhibition, HPS: Hydrogen peroxide scavenging capacity, Nd: Not determined, PAC: phenolic acids content, PMM: phosphomolybdate method, TF: Total flavonoids, TPA: Total phenolic acids, TPC: Total phenolic content.

ACKNOWLEDGEMENTS

The authors are grateful to the Algerian Ministry of Higher Education and Scientific Research for the financial support.

BIBLIOGRAPHICAL REFERENCES

[1]. Cheigh CI., Chung EY. & Chung MS. (2012). Enhanced extraction of flavanones hesperidin and narirutin from *Citrus unshiu* peel using subcritical water. *J Food Eng.*110: 472–477.

[2]. Wang, Y.C., Chuang, Y.C. & Hsu, H.W. (2008). The flavonoid carotenoid and pectin content in peels of Citrus cultivated in Taiwan. *Food Chem.* 106: 277-284.

[3]. Bocco, A., Cuvelier, M.E., Richard, H. & Berset, C. (1998). Antioxidant activity and phenolic composition of citrus peel and seed extracts. *J Agric Food Chem.* 46: 2123–2129.

[4]. Xu, G.H., Chen, J.C., Liu, D.H., Zhang, Y.H. & Ye, X.Q. (2008). Minerals, phenolic compounds, and antioxidant capacity of Citus peel extract by hot water. *J Food Sci*. 73 (1): 11-18.

[5]. Kelebek, H. (2010). Sugars organic acids phenolic compositions and antioxidant activity of Grapefruit (*Citrus paradisi*) cultivars grown in Turkey. *Ind Crops Prod*.32: 269-274.

[6]. Prieto, P., Pineda, M. & Aguilar, M. (1999). Spectrophotometric quantitation of antioxidant capacity through the formation of phosphomolybdenum complex: specific application to the determination of vitamin E. *Anal Biochem.* 269: 337-341.

[7]. Ruchet RJ., Cheng SJ. & Klauring JE. (1989). Prevention of cytotoxicity and inhibition of intracellular communication by antioxidant catechins isolated from Chinese green tea. *J Carcinogenesis.* 10:1003–1008.

[8]. Bhandari, M.R. & Kawabata, J. (2004). Organic acid phenolic content and antioxidant activity of wild yam (*Dioscorea* spp.) tubers of Nepal. *Food Chem.* 88: 163-168.

[9]. Hayat K., Zhang X. & Chenc H. (2010). Liberation and separation of phenolic compounds from citrus mandarin peels by microwave heating and its effect on antioxidant activity. *Sep Purif Technol.* 73: 371–376

[10]. Choi MY., Chai C., Parka JH., Jeongmi-LEE JL. & Kwon SW. (2011). Effects of storage period and heat treatment on phenolic compound composition in dried Citrus peels (Chenpi) and discrimination of Chenpi with different storage periods through targeted metabolomic study using HPLC-DAD analysis. *J Pharm Biomed Anal*. 54: 638–645

[11]. Londoño-Londoño J., Rodrigues De Lima V., Lara O. & Gil A. (2010). Clean recovery of antioxidant flavonoids from citrus peel: Optimizing an aqueous ultrasound-assisted extraction method. *Food Chem.* 119: 81–87

[12]. Chen ZT., Chu HL., Chyau CC., Chu CC. & Duh PD. (2012). Protective effects of sweet orange *(Citrus sinensis*) peel and their bioactive compounds on oxidative stress. *Food Chem.* 135: 2119–2127

[13]. Manthey JA. & Grohmann K. (1996). Concentrations of hesperidin and other orange peel flavonoids in citrus Processing By-products. *J Agric and Food Chem.* 44: 811-814

[14]. Sawalha SMS., Arráez-Román D., Segura-Carretero A. & Fernández-Gutiérrez A. (2009). Quantification of main phenolic compounds in sweet and bitter orange peel using CE-MS/MS. *Food Chem.*116: 567-574.

[15]. Kim, H.G., Kim, G.S., Lee, J.H., Park, S., Jeong, W.Y., Kim, Y.H., et al. (2011). Determination of the change of flavonoid components as the defence materials of *Citrus unshiu* Marc fruit peel against *Penicillium digitatum* by liquid chromatography coupled with tandem mass spectrometry. *Food Chem.* 128: 49–54.

[16]. Menichini, F., Loizzo, M.R., Bonesi, M., Conforti, F., De Luca, D., Statti, G.A., et al. (2011). Phytochemical profile antioxidant anti-inflammatory and hypoglycemic potential of hydroalcoholic extracts from *Citrus medica* L. cv Diamante flowers leaves and fruits at two maturity stages. *Food Chem Toxicol*. 49: 1549-1555.

[17]. De Lourdes MBM., Andres-Lacueva C., Jauregui O. & Lamuela-Raventos RM.(2007). Determination of flavonoids in a Citrus fruit extract by LC–DAD and LC–MS. *Food Chem.* 101: 1742–1747

[18]. Ortun˜o A., Garci´A-Puig D., Fuster MD., Pe´Rez ML., Sabater F. & Porras I.(1995) Flavanone and nootkatone levels in different varieties of grapefruit and pummel. *J Agric Food Chem.* 43: 1–5.

[19]. Castillo J., Benavente O. & Del-Rio JA. (1993). Hesperetin 7-O-glucoside and prunin in Citrus species *(C aurantium* and *C paradisi*) A study of their quantitative distribution in immature fruits and as immediate precursors of neohesperidin and naringin in Citrus aurantium. *J Agric Food Chem.* 41: 1920–1924.

[20]. Hsu, C.Y. (2006). Antioxidant activity of extract from *Polygonum aviculare* L. *Biol Res.* 39: 281-288.

[21]. Ris-Etherton, P.M., Hecker, K.D., Bonanome, A., Coval, S.M, Binkoski, A.E., Hilpert, K.F., et al. (2002). Bioactive compounds in foods: Their role in the prevention of cardiovascular disease and cancer. *Am J Med.* 113(9): 71–88.

[22]. Rice-Evans CA., Miller NJ., Bolwell PG., Bramley PM. & Pridham JB. (1995). The relative antioxidant activities of plant-derived polyphenolic flavonoids. *Free Radical Res.* 22:375-383.

[23]. Z˘ilic´, S.S.,˘UKALOVIC´, V.H.T, Dodig, D., Maksimovic´, V. & Maksimovic´, M. (2011). Antioxidant activity of small grain cereals caused by phenolics and lipid soluble antioxidants. *J Cereal Sci*. 54: 417–424.