JOURNAL OF AGRICULTURAL AND FOOD CHEMISTRY

ARTICLE

pubs.acs.org/JAFC

Role of the Cultivar in Choosing Clementine Fruits with a High Level of Health-Promoting Compounds

Luigi Milella, Marisa Caruso, Fernanda Galgano,* Fabio Favati, Maria Carmela Padula, and Giuseppe Martelli

Dipartimento di Biologia DBAF, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy

ABSTRACT: Thirteen cultivars and two hybrids of Clementine fruits (*Citrus clementina* Hort. Ex. Tan) cultivated in Italy were characterized according to pH, titratable acidity, total soluble solids, total polyphenols, carotenoids, vitamin C, hesperidin, rutin, narirutin and naringin and radical scavenging activity. The presence of rutin in Clementine fruit juice is reported for the first time here. The results indicated that all chemical parameters statistically differentiated each cultivar ($P < 0.001$). In particular, principal component analysis showed a clear discrimination of five cultivars from all the other varieties based on vitamin C and total polyphenols for the Caffin cultivar, which showed also the highest antioxidant activity; narirutin for the Etna hybrid cultivar; hesperidin, rutin and total soluble solids for the SRA 89 cultivar; and naringin, hesperidin and rutin for the Esbal cultivar. Moreover, the Mandalate hybrid cultivar showed the lowest antioxidant activity as well as vitamin C and total polyphenols content, while titratable acidity and naringin level were the highest. The antioxidant activity assessed in all the fruits was closely correlated with vitamin C and total polyphenols content, rather than with the flavonoid compounds.

KEYWORDS: antioxidant activity, β-carotene, *Citrus clementina*, heath-promoting compounds, polyphenols, vitamin C

INTRODUCTION

The increasing obesity problems among adults and children require, as a basic approach, the consumption of a more balanced diet. The availability of snacks based on fresh fruit and vegetables, both in cafeterias and in automatic vending machines, could represent a good starting point. Clementine mandarin fruits, belonging to the genus *Citrus*, may represent a good choice in this respect, as nowadays consumers are increasingly demanding easy to eat fresh fruits, having specific characteristics such as appealing color, easy to peel rind, balanced sugar/acid ratio, firmness, and lack of seeds. Furthermore, there is strong biomedical interest in *Citrus* fruits, because a diet rich in them seems to be associated with lower risk of colorectal, esophageal, gastric and stomach cancers.[1] The negative role played by free radicals in human health is well-known, due to the fact that these moieties may induce the development of several diseases, such as cancer, hypertension, stroke and diabetes. Free radicals are originated during body metabolism, and their presence in blood can be neutralized by regular intake of foods containing a high content of antioxidants, such as fruits and vegetables. In particular, *Citrus* spp. fruits possess a high antioxidant activity, being rich in various bioactive compounds acting as free radical scavengers and exerting different antiradical activities. In addition, it has been demonstrated that regular intake of mandarin juice for 4 weeks exerts a significant positive effect on oxidation biomarkers and enhances the antioxidant defenses of hypercholesterolemic children.[2]

The health-promoting compounds responsible of these beneficial effects are vitamin C and flavonoids. Vitamin C is considered one of the most important antioxidants because of its activity against free radicals, carcinogenesis and cardiovascular diseases, as well as in stimulating the human immune system.[3] Flavonoids act as free radical scavengers, inhibit cellular proliferation, can act against high blood pressure or high levels of cholesterol and have antibiotic, antiallergic, antidiarreha, antiulcer, and anti-inflammatory activities.[4] Flavonoids are a group of polyphenolic compounds present in *Citrus* spp. as flavanones (e.g., hesperidin, narirutin and naringin), flavones (e.g., rutin) and flavonols (e.g., quercetin).[5] Flavanones, usually occurring as glycosides, constitute the majority of flavonoids in *Citrus* fruits, and these moieties are found in a limited number of fruits, each one characterized by a typical flavanone glycoside pattern. For example, naringin predominates in the juice of sour orange, while in the juice of sweet orange, hesperidin is usually accompanied by narirutin. Flavanone glycosides, such as hesperidin and naringin, possess antioxidant, blood lipid lowering and anticarcinogenic activities. In particular, hesperidin improves venous tone, enhances microcirculation and is used for the treatment of chronic venous insufficiency, while naringin inhibits selected cytochrome P-450 enzymes resulting in drug interactions.[5] Moreover, some studies have suggested that narirutin may be an effective tool in the treatment of bronchial asthma.[6]

Quercetin is another important flavonoid with demonstrated anticarcinogenic and antiarthritic properties.[7] Quercetin glycoside is mostly present as rutin in *Citrus* fruits, even if the glycosylation of flavonoids reduces their activity with respect to the corresponding aglycons.[8] However, as for other fruits, the chemical composition of *Citrus* fruits is a function of several factors, such as cultivar, genotypic differences, ripening stage, area of production and agricultural practices. In particular, a strong correlation between flavonoid content and genetic characteristics has been observed in *Citrus* fruits.[9]

Taking into account the edible characteristics of Clementine fruits, the typical sweet taste, the bright orange color and the easiness of consumption, these fruits may attract consumers of all

Received: December 29, 2010
Accepted: April 19, 2011
Revised: March 25, 2011
Published: April 19, 2011

ages, representing a valuable source of healthy molecules. However, while several authors have reported the chemical composition of different cultivars of mandarin fruits,[6,9−15] Clementine fruits have been rarely investigated.[6,9,13] In order to investigate the potentiality of Clementine fruits as a healthy snack alternative to junk food, in this study 13 cultivars and 2 hybrids of Clementine fruits (*Citrus clementina* Hort. Ex. Tan) cultivated in Italy were characterized according to pH, titratable acidity and total soluble solids. Antioxidant molecules, such as polyphenols, carotenoids, vitamin C, hesperidin, narirutin, naringin, and rutin, and radical scavenging activity, were also quantified to understand their contribution to the overall bioactive properties and to classify Clementine fruits with respect to cultivar using a multivariate statistical procedure.

MATERIALS AND METHODS

Sampling. All fruits were grown in Southern Italy on the same farm with identical soil, irrigation, and illumination conditions (Vivaio Di Natale, Metaponto, Basilicata region). The rootstock was the same for all mandarin trees, Citrange Troyer (*Citrus sinensis* Osbeck × *Poncirus trifoliata* Raf.), and all selected trees were approximately 11 years old and free of diseases.

Thirteen cultivars of Clementine mandarins (*Citrus clementina* Hort. Ex. Tan) [Caffin (CAF); Corsica 2 (COR); Esbal (ESB); Fedele (FED); Isa (ISA); Oronules (ORO); Precoce di Massafra (MAS); Ragheb (RAG); Rubino (RUB); Spinoso (SPI); SRA 63 (RA 63); SRA 89 (RA 89); SRA 92 (RA 92)] and two hybrids [Etna (ETNA) (*Citrus unshiu* × *Citrus clementina*) and Mandalate (MAN) obtained from Fortune mandarin (*Citrus clementina* × *Citrus tangerina* Hort. ex Tan.) × Avana Mandarin (*Citrus deliciosa* Tan.)] were investigated. Sampling of the fruits was carried out in December 2009, when the Clementine mandarins reached typical commercial size; 8 fruits where harvested for each cultivar, sampling 4 fruits from 2 trees. The Clementine mandarins were squeezed, and the collected juices were immediately analyzed.

Analyses. The pH was measured using a pH meter (model SA720, Orion, Milano, Italy). Titrable acidity (TA) was determined by means of titration with NaOH 0.1 mol/L until pH 8.1, and expressed as grams of anhydrous citric acid per 100 g of sample.[16] Total soluble solids (TSS) content was assessed using an Abbe refractomer (Carl Zeiss, Jena, Germany) calibrated against sucrose and expressed as °Brix.[16]

For vitamin C extraction, 1 mL of juice fruit was transferred to a 10 mL amber volumetric flask and brought up to volume using a solution of 20 mM NaH_2PO_4 acidified to pH 2.1 with 1 N HCl. The flask was then stirred five times for 5 s and the extract filtered through a 0.2 μm cellulose acetate filter (Grace, Milano, Italy) and stored in the dark at −18 °C in an amber vial, under nitrogen, until ready for analysis. Vitamin C content was assessed by HPLC according to the method proposed by Galgano et al.[17] Detection of the compound was carried out at 240 nm, checking the linearity of the UV detector over vitamin C concentrations ranging from 25.6 to 256.0 μg/mL.

Carotenoid extraction was performed according to Navarro et al.:[14] 5 mL of fruit juice was vortexed with 20 mL of acetone:hexane (4:6) and stirred for 30 s. An aliquot of the upper organic phase was then taken for measurement of the optical density at 663, 645, 505, and 453 nm in a CARY 1E UV−vis spectrophotometer (Varian, Leini, Italy). Total carotenoid content was expressed as mg of β-carotene/L of juice, according to the Nagata and Yamashita[18] equation.

Extraction of flavonoids was conducted according to the method proposed by Careri et al.,[7] while their content was assessed by HPLC according to Mouly et al.,[19] modifying the gradient elution (Table 1). Detection of the compounds was carried out at 285 nm for hesperidin, narirutin and naringin, and 260 nm for rutin, checking the linearity of the UV detector for flavonoid levels ranging from 0.5 to 20.0 μg/mL. The eluted moieties were tentatively identified according to their retention time and by spiking the samples with known amounts of standards.

Table 1. HPLC Elution Program for Flavonoids Analysis in Clementine Fruits

time (min)	A[a] (%)	B[a] (%)
0	90	10
2	87	13
22	54	46
26	35	65
38	35	65
42	90	10
50	90	10

[a] A = water/acetic acid (96:4, v/v). B = methanol.

Assessment of the total polyphenols (TPH) content required an initial extraction step: 3 mL of juice was lyophilized and extracted twice with 10 mL of methanol. The supernatants were combined and concentrated to dryness at room temperature under a stream of nitrogen. The solid residue was then dissolved in 3 mL of methanol, and then solid-phase extraction (SPE) was used to remove sugars according to the method described by Heinonen et al.[20] The extracts were used for assessing TPH content and radical scavenging activity. The amount of total polyphenols was determined according to the Folin−Ciocalteu procedure[21] by adding 200 μL of the prepared purified extract to 1.0 mL of Folin−Ciocalteu reagent and 0.8 mL of a sodium carbonate aqueous solution (7.5% w/w). The mixture was then stirred and allowed to stand in the dark for 30 min. Absorption at 723 nm was measured using a CARY 1E UV−vis spectrophotometer (Varian, Leini, Italy). TPH content was expressed as mg of gallic acid equiv (GAE)/mL of juice.

The DPPH assay was used to measure radical scavenging activity of the juices. Samples were tested individually at three different concentrations by addition to a methanolic solution of 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical (100 μM). The mixtures were stirred and allowed to stand in the dark for 30 min at 25 °C. The absorbance of the resulting solutions was measured at 517 nm against a blank sample without DPPH.[22] Results were first expressed as the half maximal inhibitory concentration (IC50). IC50 value denotes the volume (μL) of juice extract required to scavenge 50% of DPPH free radicals. In all experiments the IC50 of rutin was also determined and used as a reference in order to assess the antioxidant activity, that was expressed as mg of rutin equiv (RE)/mL of Clementine juice (J) (mg RE/mL J).

Each sample was prepared and analyzed in duplicate.

Reagents. All chemicals were of suitable analytical grade and purchased from Carlo Erba (Milano, Italy). Folin−Ciocalteu reagent, DPPH radical, ascorbic acid, hesperidin (HESP), naringin (NAR) and rutin (RUT) standards were obtained from Sigma (Milano, Italy), while narirutin (NAT) was purchased from Extrasynthese (Genay Cedex, France).

Statistical Analysis. 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 ($P < 0.05$). Principal component analysis (PCA) of the obtained data was used to highlight any cultivar differentiation of Clementine fruits. Correlation analysis was also applied to the data.

All statistical procedures were computed using the statistical package Statistica for Windows (ver. 5.1., 1997) (Statsoft Inc., Tulsa, OK, USA).

RESULTS AND DISCUSSION

LSD Test and Analysis of Variance (ANOVA). The results of Clementine fruit quality parameters are reported in Table 2. ANOVA showed that all the chemical parameters statistically

Table 2. Effect of Cultivar on Clementine Fruit Quality Parameters[a]

	cultivar													
chem params	MAS	ORO	SPIN	RA63	FED	RUB	RAG	CAF	ETNA	COR	ISA	ESB	MAN	RA92	RA89	*F*
TA (%)	2.27 bc	1.68 d	1.88 cd	2.36 bc	2.71 b	2.38 bc	2.50 b	2.35 bc	2.41 bc	3.24 b	3.42 bc	2.54 b	8.75 a	2.74 b	2.45 b	78.65***
antioxidant act. (mg RE/mL J)	16.48 cde	15.68 cde	16.38 cd	15.79 cde	21.61 ef	17.63 def	21.23 ef	26.48 f	12.90 bc	15.44 cde	11.74 b	19.23 def	6.77 a	17.43 cde	18.56 def	14.67***
β-carotene (mg/L)	16.28 ab	11.84 gh	12.16 fgh	14.35 bcde	10.96 h	13.71 cdefg	15.77 abc	12.32 efgh	14.31 bcdef	12.91 defgh	13.76 cdefg	14.74 bcd	11.19 h	11.89 gh	17.54 a	6.46***
TSS (%)	11.12 abcd	9.87 efg	9.87 efg	11.25 abcd	10.62 cdef	9.25 g	11.12 abcd	11.37 abc	10.25 defg	10.87 bcde	11.25 abcd	9.62 fg	11.50 abc	12.12 a	11.75 ab	5.56***
HESP (mg/L)	111.92 de	125.3 dc	106.9 de	123.73 dc	126.54 cd	165.81 a	88.71 ef	134.27 bcd	63.98 f	164.88 a	117.87 d	148.56 abc	147.62 abc	150.09 abc	161.82 ab	8.91***
NAR (mg/L)	0.46 cde	0.73 cde	1.16 bc	0.60 cde	0.15 de	0.74 cde	0.60 cde	0.02 e	1.72 b	1.29 bc	1.30 bc	2.70 a	2.70 a	0.45 cde	1.02 bcd	6.71***
NAT (mg/L)	6.71 d	16.32 c	26.78 b	7.14 d	8.27 d	6.15 d	19.00 c	5.58 d	43.17 a	9.96 d	6.00 d	10.15 d	19.71 c	10.39 d	9.12 d	24.44***
pH	3.52 bc	3.75 a	3.58 b	3.37 cdef	3.35 def	3.49 bc	3.41 cde	3.36 cdef	3.43 bcd	3.24 f	3.42 cd	3.25 f	2.60 g	3.44 bcd	3.25 ef	20.13***
RUT (mg/L)	50.70 def	54.46 cde	45.73 ef	54.20 cde	56.21 bcde	75.15 a	38.75 fg	62.25 abcd	29.48 g	65.48 abc	64.27 abcd	61.72 abcd	70.98 a	68.97 ab	70.31 ab	6.41***
TPH (mg/L)	359.71 de	295.49 ef	312.9 ef	330.50 def	453.96 bc	332.89 def	367.17 de	554.55 a	320.63 ef	486.50 ab	324.25 def	364.29 de	269.19 f	332.71 def	395.97 cd	8.77***
vit C (mg/L)	607.45 bc	484.11 d	641.99 ab	632.18 b	643.73 ab	567.61 bcd	621.33 b	754.91 a	476.04 d	568.56 bcd	499.22 cd	502.34 dc	205.85 e	605.28 bc	604.84 bc	9.53***

[a] *F*: calculated Fisher's *F*. Values are the mean of 8 determinations. Data in the same row with different letters are significantly different (LSD test at $P < 0.05$). ***: Significant for $p < 0.001$.

differentiated the fruits according to cultivar ($P < 0.001$). In particular, pH values ranged from 3.24 for COR cultivar to 3.75 for ORO cultivar and were in line with those reported in the literature for mandarin fruits,[12] with the exception of the MAN cultivar fruits (pH = 2.60), which showed also the highest titratable acidity value (8.75%).

The mean content of β-carotene found in all the studied fruits was 13.58 mg/L, ranging from 10.96 mg/L for FED cultivar to 17.54 mg/L for RA89 cultivar. All Clementine varieties analyzed showed a high content of this bioactive compound with respect to that reported in other studies. In Clementine SRA 85 and in Mandarin SRA 133 cultivars the β-carotene content found by Dhuique-Mayer et al.[13] was 1.45 and 1.60 mg/L, respectively, while in the juice of two hybrids obtained from Clementine × orange, Rapisarda et al.[23] found a total carotenoid content, expressed as β-carotene, of 4.34 and 28.73 mg/L.

In comparison with other commercial citrus juices, the high level of carotenoids found in Clementine juice is of interest from a nutritional point of view, the assessed carotenoid content being more than twice that reported for commercial orange juices.[24]

With regard to TPH, the level in orange fruits has been generally reported to range from 50 to 100 mg/100 g fresh weight,[25] while for mandarin fruits, Navarro et al.[14] have reported a TPH level of 560 mg/L, a value similar to that found in the present study for the CAF cultivar (554 mg/L). However, this value was the highest observed, showing for all the other cultivars a TPH content ranging from 259 to 487 mg/L.

In the literature, the presence of vitamin C in mandarin juice has been reported as ranging from 400 to 480 mg/L,[11,13,26] while in juices produced from Clementine fruits and hybrids obtained from Clementine × orange this vitamin has been found at higher concentrations, ranging from 500 to about 580 mg/L.[13,23] In a recent study of Navarro et al.,[14] relatively high levels of vitamin C (612 mg/L) in mandarin fruits have been reported, and this value is similar to that found in some of the studied Clementine cultivars (MAS, ORO, SRA 63, FED, RUB, RAG, CORS, RA 92 and RA 89). In addition, the vitamin C content assessed in the ORO cultivar (484 mg/L) is similar to that reported for this variety by Cano et al.[9] (474 mg/L). Only the cultivars CAFFIN and MAN exhibited a significantly different vitamin C concentration, whose value was shown to be the highest (755 mg/L) in the former and the lowest (206 mg/L) in the latter.

The Experts Committee of the European Fruit Juice Association (AIJN) has established as a quality parameter a minimum level of 300 mg/L of vitamin C for orange and mandarin juices.[27] In the present study, with the exception of the MAN cultivar, one of the two hybrids analyzed, all the fruits showed a vitamin C level much higher than the minimum content set by AIJN, and the result is therefore of interest for choosing Clementine fruits with a high level of this health-promoting compound (Table 2).

In *Citrus* fruits flavanone glycosides are normally present in decreasing sequence as follows: HESP > NAT > NAR. In particular, in orange juices the concentration of these moieties has been reported as spanning over a wide range, namely, from 107 to 760 mg/L for HESP, from 35 to 194 mg/L for NAT and from 14 to 45 mg/L for NAR, according to fruit variety and ripening stage.[7,19,28,29] Several studies have reported that in mandarins HESP represents the main flavanone glycoside, followed by NAT, found at lower concentrations, while the presence of NAR has not been reported in mandarin,[1,7,8] this moiety being typically found in sour oranges and present in sweet oranges only at low concentrations.[1]

Table 3. Pearson Correlation Coefficients (r) and Related Significance between Antioxidant Content and Antioxidant Activity

	antioxidant activity	
	r	P
β-carotene	0.08	ns[a]
HESP	0.02	ns
NAR	−0.18	ns
NAT	−0.21	ns
RUT	−0.10	ns
TPH	0.63	<0.001
vit C	0.71	<0.001

[a] No significant correlation.

For HESP, the concentrations assessed in the tested Clementine fruits (63−165 mg/mL juice) are in agreement with those reported by Kanaze et al.[5] (52−166 mg/mL juice) and Levaj et al.[6] (62 mg/kg of pulp). As far as NAT, the concentration reported in the literature for mandarin and Clementine fruits (40 mg/kg of pulp)[6] is within the range found in this study (6−43 mg/L of juice). In the same paper, the authors have reported a very high NAR average content (31 mg/kg of pulp), while our experimental data indicate a much lower NAR content in all the investigated fruits, ranging from 0.02 to 2.70 mg/L. However, also Kanaze et al.[5] have reported low levels of NAR in mandarin fruit juice (average value 0.8 mg/L).

Quercetin is another important compound with demonstrated anticarcinogenic and antiarthritic properties for human health.[7] In particular, quercetin has been shown to mediate the downregulation of mutant p53 in a human breast cancer cell line.[8] Quercetin glycoside is mostly present as RUT in orange juice at levels of 29 mg/kg fresh weight,[30] and in the present study RUT was found in the juice of all Clementine varieties, ranging from 29.48 for ETNA to 75.15 mg/L for RUB cultivar. To our knowledge, this is the first study reporting the presence of rutin in Clementine fruits; some authors have found this nutrient in flavedo extracts of Mauritian Mandarin and Clementine fruits at an average level of 28.75 mg/g fresh weight,[31] while Wang et al.[32] found it at low levels in the peels of several citrus cultivated in Taiwan (0.25 mg/g dried matter). The presence of RUT in the juices could be to some extent ascribed to the extraction of this moiety from the pulp during the squeezing process. However, the assessed levels are quite high, so that inferring the presence of RUT in the pulp, and consequently in the juice, is reasonable. Despite this, further investigation is needed on the same cultivars to confirm the presence of RUT in juice produced from individually and manually peeled mandarin segments.

Several studies have reported correlations between bioactive compounds and antioxidant activity in several *Citrus* fruits.[10,12,13,33−36] However, no information is present in the literature regarding Clementine fruits. Generally in *Citrus* fruits, the antioxidant activity is attributed to hydrophilic compounds, and some authors have commented that HESP is the principal compound responsible for the total antioxidant capacity of orange juices.[13]

In this study, significant correlations were observed between vitamin C and antioxidant activity for Clementine fruits ($r = 0.71$) and between total polyphenol content and antioxidant activity ($r = 0.63$) (Table 3). On the other hand, no significant correlation between individual flavonoid content and antioxidant activity or

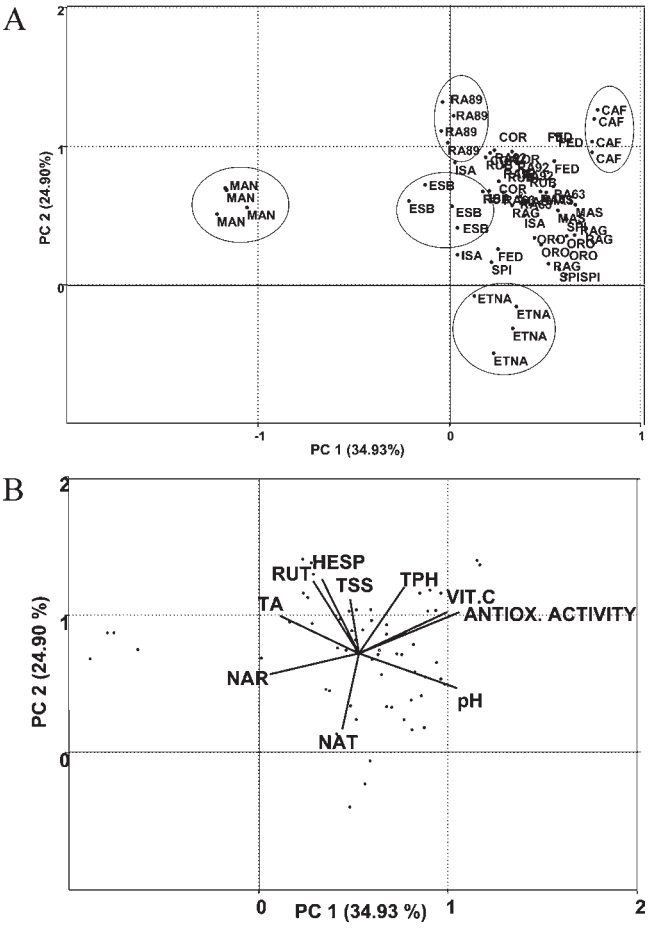

Figure 1. Score plot (A) and loading plot (B) of the first (PC1) and second (PC2) principal components obtained by principal component analysis of the chemical profile of Clementine fruits (13 cultivars and 2 hybrids).

between β-carotene and antioxidant activity was found. Moreover, this correlation was negative for NAT, RUT and NAR.

Several authors have reported that the antioxidant activity exerted by *Citrus* fruits, such as oranges and lemons, is principally due to the concentration of vitamin C and phenolic compounds, while flavonoids do not contribute significantly to the antioxidant potential of antioxidant compounds, even if the latter are present in great quantity in *Citrus* fruits.[4,10,12,35,36] The lack of correlation between flavonoid compounds and antioxidant activity could also be due to the synergistic effect among all the hydrophilic antioxidants, as also reported for tomato antioxidants.[37] Negative correlations between flavonoid content and antioxidant activity have also been reported by Meda et al.[34] and Ghafar et al.,[33] probably because of the presence of other phenolics in *Citrus* fruits which might have contributed to the antioxidant activity. Conversely, in mandarin fruits Levaj et al.[6] have reported a high correlation between total flavonoids, as well as individual flavanone glycosides (narirutin, naringin and hesperidin), and antioxidant capacity.

Principal Component Analysis. In order to discriminate the fruits on the basis of the principal chemical quality parameters, the data were normalized and PCA was conducted on the correlation matrix. After the variable (β-carotene) with low statistical loading (<0.25) was removed, the new data matrix obtained was subjected to PCA and the first two principal

components, with eigenvalues greater than unity, were selected, accounting for 59.83% of the total variance, 34.93% of which along PC1 and 24.90% along PC2. The results related to the chemical profile of Clementine fruits obtained from 13 cultivars and 2 hybrids are shown in Figure 1, where the score plot and the loading plot of the variables are reported, respectively.

Besides vitamin C, TSS, TA and TPH, flavonoids were useful for discriminating Clementine fruits according to cultivar, as also observed for other citrus species.[9,38] Conversely, β-carotene content was not a useful marker to differentiate Clementine fruits according to variety, in contrast to other studies where citrus fruits were discriminated on the basis of the carotenoid content.[13] In particular, the PCA showed a clear discrimination of five cultivars from all the other varieties based on the following parameters: vitamin C and TPH for CAF cultivar, which showed also the highest antioxidant activity; NAT for ETNA hybrid cultivar; HESP, RUT and TSS for SRA 89 cultivar; NAR, HESP, and RUT for ESB cultivar. Moreover, fruits belonging to MAN hybrid cultivar showed the lowest antioxidant activity as well as vitamin C and TPH content, while the TA and NAR levels were the highest.

In conclusion, the results of this study highlighted that health-promoting compounds, besides TA, TSS and pH, have potential for discriminating Clementine fruits by cultivar. This information could be useful for choosing varieties with a high content of nutraceutical moieties. In particular, PCA of the analytical data showed a clear discrimination of five cultivars from all the other varieties based on the following parameters: vitamin C and TPH for CAF cultivar which also had the highest antioxidant activity; NAT for ETNA hybrid cultivar; HESP, RUT and TSS for SRA 89 cultivar; NAR, HESP, and RUT for ESB cultivar. Furthermore, fruits belonging to MAN hybrid cultivar showed the lowest antioxidant activity as well as vitamin C and TPH content, while TA and NAR levels were the highest.

As far as the antioxidant properties of the various juices tested are concerned, of interest is the fact that the antioxidant activity was clearly correlated with vitamin C and total polyphenols rather than with the amount of flavonoid compounds.

AUTHOR INFORMATION

Corresponding Author

*Tel: +39 0971 205570. Fax: +39 0971 205503. E-mail: fernanda.galgano@unibas.it.

ACKNOWLEDGMENT

The authors would like to thank Piergiovanni Lardo and Daniela Russo for their skillful technical assistance. The authors would like to thank Consorzio Covil, Vivaio Di Natale and Dr. Carmelo Mennone for supplying the samples.

REFERENCES

(1) Peterson, J. J.; Dwyer, J. T.; Beecher, G. R.; Bhagwat, S. A.; Gedhardt, S. E.; Haytowitz, D. B.; Holden, J. M. Flavanones in oranges, tangerines (mandarins), tangors, and tangelos: a compilation and review of the data from the analytical literature. *J. Food Compos. Anal.* **2006**, *19*, S66–S73.

(2) Codoñer-Franch, P.; López-Jaén, A. B.; Muñiz, P.; Sentandreu, E.; Bellés, V. V. Mandarin juice improves the antioxidant status of hypercholesterolemic children. *J. Pediatr. Gastroenterol. Nutr.* **2008**, *47*, 349–355.

(3) Galgano, F.; Favati, F.; Caruso, M.; Pietrafesa, A.; Natella, S. The Influence of processing and preservation on the retention of health-promoting compounds in broccoli. *J. Food Sci.* **2007**, *72*, 499–504.

(4) Yu, J.; Wang, L.; Walzem, R. L.; Mileer, E. G.; Pike, L. M.; Patil, B. S. Antioxidant activity of citrus limonoids, flavonoids, and coumarines. *J. Agric. Food Chem.* **2005**, *53*, 2009–2014.

(5) Kanaze, F. I.; Gabrielli, C.; Kokkalou, E.; Georgarakis, M.; Niopas, I. Simultaneous reversed-phase High-performance liquid chromatographic method for the determination of diosmin, naringin and hesperidin in different citrus fruit juices and pharmaceutical formulation. *J. Pharm. Biomed. Anal.* **2003**, *33*, 243–249.

(6) Levaj, B.; Dragović-Uzelac, V.; Bursać Kovačević, D. B.; Krasnići, N. Determination of flavonoids in pulp and peel of mandarin fruits. *Agric. Conspectus Scientificus* **2009**, *74*, 221–225.

(7) Careri, M.; Elviri, L.; Mangia, A.; Musci, M. Spectrometric and coulometric detection in the high-performance liquid chromatography of flavonoids and optimization of sample treatment for the determination of quercetin in orange. *J. Chromatogr., A* **2000**, *881*, 449–460.

(8) Rice-Evans, C.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20*, 933–956.

(9) Cano, A.; Medina, A.; Almudena, Bermelo, A. (2008). Bioactive compounds in different citrus varieties. Discrimination among cultivars. *J. Food Compos. Anal.* **2008**, *21*, 377− 381.

(10) Abeysinghe, D. C.; Li, X.; Sun, C.; Zhang, W.; Zhou, C.; Chen, K. Bioactive compounds and antioxidant capacities in different edible tissues of citrus fruit of our species. *Food Chem.* **2007**, *104*, 1338–1344.

(11) Beltràn, F.; Perèz Lòpez, A. J.; Lòpez-Nicolàs, J. M.; Carbonell-Barachina, A. A. Effects of mandarin Cultivar on Quality of mandarin juice. *Food Sci. Technol. Int.* **2008**, *14*, 307–313.

(12) Del Caro, A.; Piga, A.; Vacca, V.; Agabbio, M. Changes of flavonoids, vitamin C and antioxidant capacity in minimally processed citrus segments and juices during storage. *Food Chem.* **2004**, *84*, 99–105.

(13) Dhuique-Mayer, C.; Caris-Veyrat, C.; Ollitrault, P.; Curk, F.; Amiot, M. J. Varietal and interspecific influence on micronutrient contents in citrus from the Mediterranean area. *J. Agric. Food Chem.* **2005**, *53*, 2140–2145.

(14) Navarro, J. M.; Pèrez-Pèrez, J. G.; Romero, P.; Bota, P. Analysis of changes in quality in mandarin fruit, produced by deficit irrigation treatments. *Food Chem.* **2010**, *119*, 1591–1596.

(15) Xu, G.; Liu, D.; Jianchu Chen, J.; Ye, X.; Shi, J. Composition of major flavanone glycosides and antioxidant capacity of three citrus varieties. *J. Food Biochem.* **2009**, *33*, 453–469.

(16) AOAC. *Official methods of analysis of AOAC: food composition; additives; natural contaminants*; Helrich, K., Ed.; AOAC: Arlington, 1990; Vol. II.

(17) Galgano, F.; Favati, F.; Lapelosa, L.; Albanese, D.; Montanari, L. Effect of chilling on the vitamin C content of fennel during storage. *Ital. J. Food Sci.* **2002**, *14*, 167–173.

(18) Nagata, M.; Yamashita, I. Simple method for simultaneous determination of chlorophyll and carotenoids in tomato fruits. *J. Jpn. Soc. Food Sci. Technol.* **1992**, *39*, 925–928.

(19) Mouly, P.; Emile, M.; Gaydou, E. M.; Auffray, A. Simultaneous separation of flavanone glycosides and polymethoxilated flavanones in citrus juices using liquid chromatography. *J. Chromatogr., A* **1998**, *800*, 171–179.

(20) Heinonen, I. M.; Lehtonen, P. J.; Hopia, A. I. Antioxidant activity of berry and fruit wines and liquors. *J. Agric. Food Chem.* **1998**, *46*, 25–31.

(21) Singleton, V. L.; Rossi, J. A. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* **1965**, *16*, 144–158.

(22) Bonina, F.; Saija, A.; Tomaino, A.; Lo Cascio, R.; Rapisarda, P.; Dederen, J. C. In vitro antioxidant activity and in vivo photoprotective effect of a red orange extract. *Int. J. Cosmet. Sci.* **1998**, *20*, 331–342.

(23) Rapisarda, P.; Bellomo, S. E.; Fabroni, S.; Russo, G. Juice quality of two new mandarin-like hybrids (*Citrus clementina* Hort. Ex tan x *Citrus sinensis* L. Osbeck) containing anthocyanins. *J. Agric. Food Chem.* **2008**, *56*, 2074–2078.

(24) Gardner, P. T.; White, T. A. C.; McPahil, D. B.; Duthie, G. G. The relative contributors of vitamin C, carotenoids and phenolics to the antioxidant potential of fruits juices. *Food Chem.* **2000**, *68*, 471–474.

(25) Wang, Y. C.; Chuang, Y. C.; Ku, Y. H. Quantitation of bioactive compounds in citrus fruits cultivated in Taiwan. *Food Chem.* **2007**, *102*, 1163–1171.

(26) Pèrez-Lòpez, A. J.; Carbonell-Barrachina, A. A. Volatile odour components and sensory quality of fresh and processed mandarin juices. *J. Sci. Food Agric.* **2006**, *86*, 2404–2311.

(27) AIJN (European Fruit Juice Association, 2006). AIJN Code practice for evaluation of fruit and vegetable juices. Available at http:/ www.aijn.org.

(28) Cao, S.; Liu, L.; Lu, Q.; Xu, Y.; Pan, S.; Wang, K. Integrated effects of ascorbic acid, flavonoids and sugars on thermal degradation of anthocyanins in blood orange juice. *Eur. Food Res. Technol.* **2009**, *228*, 975–983.

(29) Lee, H. S.; Carter, R. D.; Barros, S. M.; Dezman, D. J.; Castle, W. S. Chemical characterization by liquid chromatography of Moro blood orange juices. *J. Food Compos. Anal.* **1990**, *3*, 1–19.

(30) Nogata, Y.; Hideaki Ohta, H.; Yoza Berthow, K.; Hasegawa, S. High-performance liquid chromatographic determination of naturally occurring flavonoids in citrus with a photodiodearray detector. *J. Chromatogr., A* **1994**, *667*, 59–66.

(31) Ramful, D.; Baharun, T.; Bourdan, E.; Tarnus, E.; Aruoma, O. I. Bioactive phenolics and antioxidant propensity of flavedo axtracts of Mauritian citrus fruits: potential prophylactic ingredients for functional foods application. *Toxicology* **2010**, *278*, 75–87.

(32) Wang, Y. C.; Chuang, Y. C.; Hsing-Wen, H. The flavonoid, carotenoid and pectin content in peels of citrus cultivated in Taiwan. *Food Chem.* **2008**, *106*, 277–284.

(33) Ghafar, A. M. F.; Prasad, K. N.; Weng, K. K.; Ismail, A. Flavonoid, hesperidine, total phenolic contents and antioxidant activities from *Citrus* species. *Afr. J. Biotechnol.* **2010**, *9*, 326–330.

(34) Meda, A.; lamien, C. E.; Romito, M.; Millogo, J.; Nacouloma, O. G. Determination of the total phenolic, flavonoid and proline contents in Burkina Fasan honey, as well as their radical scavenging activity. *Food Chem.* **2005**, *91*, 571–577.

(35) Miguel, M. G.; Duarte, A.; Nunes, S.; Sustelo, V.; Martins, D.; Dandlen, S. A. Ascorbic acid and flavanone glycosides in citrus: relationship with antioxidant activity. *J. Food Agric. Environ* **2009**, *7*, 222–227.

(36) Proteggente, A. R.; Saija, A.; De Pasquale, A.; Rice-Evans, C. A. The compositional characterization and antioxidant activity of fresh juices from Sicilian sweet orange (*Citrus sinensis* L. Osbeck) varieties. *Free Radical Res.* **2003**, *37*, 681–687.

(37) Lenucci, M. S.; Cadinu, D.; Taurino, M.; Piro, G.; Dalessandro, G. Antioxidant composition in cherry and high-pigment tomato cultivars. *J. Agric. Food Chem.* **2006**, *54*, 2606–2613.

(38) Mouly, P. P.; Arzouyan, C. R.; Gaydou, E. M.; Estienne, J. M. Differentiation of citrus juices by factorial discriminant analysis using liquid chromatography of flavanone glycosides. *J. Agric. Food Chem.* **1994**, *42*, 70–79.