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Varietal and Interspecific Influence on Micronutrient Contents in Citrus from the Mediterranean Area

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To specify the genotypic variation of Mediterranean *Citrus* juices, the contents of carotenoids, flavonoids, and vitamin C were determined by high-performance liquid chromatography. A selection of orange varieties and Mandarin species from the Mediterranean area (*Citrus sinensis*, *Citrus deliciosa* Ten, and *Citrus clementina* Hort. ex Tan) was evaluated using carotenoid profiles and flavanones contents. Among the eight varieties of orange (Salustiana, Hamlin, Shamouti, Pera, Valencia, Maltaise, Sanguinelli, and Cara-cara) and two Mandarin species, only three cultivars (Pera, Sanguinelli, and Shamouti) and the two Mandarin species displayed a high content of vitamin A (374, 381, and 272 ER L^{-1} for the three orange cultivars and 1156 and 960 retinol equivalent (RE) L^{-1} for the Mandarins) due to a high content of β-cryptoxanthin. These same *Citrus* were also rich in hesperidin (502, 537, 552, 767, and 754 mg L^{-1}, respectively). Principal component analysis allowed the Mediterranean orange varieties and Mandarin species to be differentiated on the basis of nutritional criteria. Strong correlations were observed between β-cryptoxanthin and hesperidin ($r = 0.92$) and between β-cryptoxanthin and β-carotene ($r = 0.98$). In contrast, vitamin C content was not correlated with carotenoids and flavanone glycosides. The Mandarin and orange group was quite distinct. The orange varieties could be divided in two groups. In addition, a diversity tree allowed a genetic approach to differentiating *Citrus* cultivars on the basis of Euclidian distances. This representation showed that the hybrid Clementine was nearer to its parent Mandarin than to its parent orange, suggesting that β-cryptoxanthin was a dominant genetic factor. With regard to vitamin A, Mandarin and its hybrid Clementine appeared to be the best *Citrus* species.

KEYWORDS: ***Citrus sinensis*; *Citrus reticulata*; Mediterranean *Citrus*; micronutrients; carotenoid; hesperidin; vitamin C; β-cryptoxanthin; orange juice, varietal selection**

INTRODUCTION

Consumption of citrus fruits has been associated with reduced risks of certain cancers and cardiovascular diseases (*1*−3). Citrus juices, especially orange juice, are considered to be rich sources of antioxidants including vitamin C, phenolic compounds and carotenoids, all of them contributing to the beneficial health effects of citrus fruits and citrus-derived products (*4*, *5*). Among these phytochemicals, some authors have stressed the main role of hesperidin in the total antioxidant capacity of orange juices (*6*). Moreover, studies on the bioavailability of hesperetin in humans have shown high plasmatic concentrations of hesperetin after the ingestion of citrus juices; thus, beneficial health effects could be achieved by regular consumption (*7*, *8*). Although the antioxidant capacity of citrus juices has been mainly associated with the hydrosoluble fraction containing polyphenols and vitamin C, the more apolar fraction including carotenoids could also contribute to the antioxidant capacity of the juices, leading to their protective effects against degenerative diseases. Among carotenoids present in *Citrus*, some of them have been detected in human plasmas: α- and β-carotene, lycopene, β-cryptoxanthin, lutein, and zeaxanthin. Besides α- and β-carotene, β-cryptoxanthin, which contributes in large part to the bright orange color of juice (*9*) and is the main precursor of provitamin A in citrus juices, has been described as the major carotenoid in Mandarin with relatively high concentrations being reported in certain orange cultivars (*10*). Furthermore, Miller et al. (*11*) reported that β-cryptoxanthin was the most efficient radical scavenger after lycopene. Recently, bioavailability studies in

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humans have reported β-cryptoxanthin as a biomarker of Mandarin consumption (*12*, *13*).

In this work, we compared the varietal influence at inter- and intraspecific levels on the micronutrient contents of different citrus juices from fruit harvested in Corsica. Whereas the literature usually reported the content of only one class of phytochemicals (*6*, *14*, *15*), when we consider the nutritional value of fruit, it appears pertinent to evaluate the content of different microconstituents at the same time. In addition, these microconstituents could have synergistic effects as was previously shown (*4*, *5*). In this paper, we report the influence of variety and species on the content of the main antioxidant microconstituents present in *Citrus*, flavanones, carotenoids, and vitamin C.

MATERIALS AND METHODS

Citrus Fruits. Different varieties of oranges (*Citrus sinensis*) from Agronomic Research Station (SRA) selections in Corsica were chosen: cv. Salustiana SRA 508; cv. Hamlin SRA 41; cv. Shamouti (Jaffa) SRA 538; cv. Pera; SRA 399; cv. Valencia SRA 246; cv. Maltaise SRA 560; cv. Sanguinelli SRA 243; and Cara-cara SRA 666. The other species used were Mandarin SRA 133 (*Citrus deliciosa* Ten) and Clementine SRA 85 (C*itrus clementina* Hort. ex Tan) following the Tanaka classification. Rootstocks were *Poncirus trifoliata* (L.) Raf. cv. Pomeroy. All fruits were harvested during the 2002−2003 season between December and April. The annual average temperature in Corsica in 2002 was 16 °C, the average maximum temperature being 21 °C and the average minimum temperature being 11 °C. Rainfall in 2002 was 602 mm, so only 225 mm of irrigation was necessary. Representative samples (15 fruits/cultivar) were collected from three trees. First, variability was determined between fruits to calculate an appropriate sample size. A sample size of 15 fruits per cultivar was determined using a statistic bilateral test (with a first risk, $\alpha = 0.05$, and a second risk, $\beta = 0.20$). Citrus fruits were received at maturity stage (maturity index: soluble solids/titrable acidity = 7) were immediately hand-squeezed and then filtered through a stainless steel sieve (1 mm). The juices were placed in sealed amber vials (15 mL) under nitrogen and kept frozen at −20 °C until analyzed; storage time did not exceed 1 month.

Reagents. Extraction solvents were RPE grade hexane, ethanol, and dichloromethane from Carlo-Erba (Val de Reuil, France). Analytic solvents were HPLC grade methanol, acetonitrile, and tetrahydrofuran (THF), also from Carlo-Erba, and methyl *tert*-butyl ether (MTBE) from Sigma-Aldrich (Steinheim, Germany). Reagents for analyses were pure grade *N*,*N*-dimethylformamide (DMF), sodium chloride, sodium sulfate, ammonium oxalate, and metaphosphoric acid from Carlo-Erba. L-(+)-Ascorbic acid, dehydroascorbic acid, and dl-dithiothreitol were from Sigma-Aldrich (Steinheim, Germany). Standards used were purchased from Extrasynthese (Genay, France): β-carotene, β-cryptoxanthin, zeaxanthin, lutein, lycopene, β-apo-8′-carotenal, narirutin (NAT), hesperidin (HES). (The purities of standards were verified by HPLC and photodiode array detection).

Carotenoid Extraction. Carotenoid extraction was carried out according to the method of Taungbodhitham et al. (*16*). An aliquot (20 g) of orange juice was homogenized by magnetic stirrer with 120 mg of $MgCO_3$ and 35 mL of extraction solvent (ethanol/hexane, 4:3 v/v, containing 0.1% of BHT as antioxidant) for 5 min. Lycopene (750 μL of solution, equivalent to 90 μg) was added as an internal standard for all *Citrus* except for the red Cara-cara variety, in which lycopene was detected, where 150 μL of β-apo-8′-carotenal, equivalent to 40 μg, was used. The residue was separated from the liquid phase by filtration with a filter funnel (porosity no. 2) and re-extracted with 35 mL of ethanol/hexane as previously. Ethanol (30 mL) and hexane (30 mL) were successively used to wash the residue. Organic phases were transferred to a separatory funnel and washed with 2 × 50 mL of 10% sodium chloride and 3 × 50 mL of distilled water. The aqueous layer was removed. The hexanic phase was dried using anhydrous sodium sulfate and filtered before evaporation to dryness under vacuum at 40 °C. Carotenoid extracts were dissolved in 500 μL of dichloromethane and 500 μL of an 80:20 (v/v) mixture of MTBE and methanol. This solution was diluted 6-fold in the MTBE/methanol mixture and stored in amber vials before HPLC analysis.

Saponification. The hexanic extract was evaporated to dryness with a rotary evaporator, redissolved with 20 mL of hexane, and placed in a 50 mL amber vial to which was added 20 mL of 10% methanolic KOH. Saponification was carried out overnight in the dark at room temperature. The sample was shaken under nitrogen in the sealed vial. The sample was transferred to a separatory funnel to which 50 mL of distilled water was added to separate the layers. The hexanic layer was rinsed until free of alkali. The methanolic KOH layer was extracted with 3 × 15 mL of dichloromethane. The extracts were pooled and washed to remove alkali. Aqueous traces from organic extracts were removed with anhydrous sodium sulfate; then the extracts were filtered and evaporated to dryness under vacuum. Carotenoid extracts were dissolved as described above. Analyses were conducted under red light to avoid carotenoid degradation during extraction and saponification. The coefficient of variation for extraction−saponification was <5%.

HPLC Analysis of Carotenoids. Carotenoids were analyzed by reverse-phase high-performance liquid chromatography using an Agilent 1100 system (Massy, France) according to the previously published method of Caris et al. (*17*). Carotenoids were separated along a C_{30} column (250 × 4.6 mm i.d., 5 μm YMC (EUROP GmbH); the mobiles phases were H_2O as eluent A, methanol as eluent B, and MTBE as eluent C. Flow rate was fixed at 1 mL min^{-1}, column temperature was set at 25 °C, and injection volume was 20 μL. A gradient program was performed: the initial condition was 40% A/60% B; 0−5 min, 20% A/80% B; 5−10 min, 4% A/81% B/15% C; 10−60 min, 4% A/11% B/85% C; 60−71 min, 100% B; 71−72 min, back to the initial condition for reequilibration. Absorbance was followed at 290, 350, 450, and 470 nm using an Agilent 1100 photodiode array detector. Chromatographic data and UV−visible spectra were collected, stored, and integrated using an Agilent Chemstation plus software. Quantification of carotenoids was achieved using calibration curves with β-carotene, β-cryptoxanthin, lutein, lycopene, and β-apo-8′-carotenal with five concentrations. Correlation coefficients ranged from 0.994 to 0.998. Recoveries were determined by adding internal standard (lycopene or β-apo-8′-carotenal) before the extraction of each sample analyzed and used to correct carotenoids content after HPLC analysis. To verify the losses of carotenoids during extraction and saponification, carotenoid standards (β-cryptoxanthin and lycopene) were extracted and saponified. Mean losses were ∼20%. Mean recovery in juice was 84% for β-cryptoxanthin. In addition, to minimize losses during HPLC analysis, each extract was immediately injected after its preparation. In fact, losses in lycopene (alone) were evaluted as close to 10% after 12 h at low temperatures (5−6 °C).

Preparation of Standards. The concentrations of external standard solutions were determined using molar extinction coefficient (ϵ_{mol}) in appropriate solvent checked by spectrophotometry according to the method of Britton et al. (*18*). Internal standards were diluted in dichloromethane.

Vitamin A Value. The vitamin A value was calculated as retinol equivalent (RE), using the following conversion:

$$\mathrm{RE} = (\mu\text{g of } \beta\text{-carotene}/6) + (\mu\text{g of other provitamin A [}\beta\text{-cryptoxanthin] carotenoid}/12)$$

(In our case only β-carotene and β-cryptoxanthin contribute to vitamin A activity.)

Extraction and Quantification of Flavanone Glycosides (FG). The extraction method was the same as the procedure described by Mouly et al. (*19*). The HPLC analytical system was an Agilent 1100 model (Massy, France). Separation of flavanones was performed by HPLC using an RP 18e Licrospher 100 (5 μm) column (250 mm × 4.6 mm i.d.) (Merck KgaA). The isocratic solvent system was water/acetonitrile/THF/acetic acid (80:16:3:1, v/v/v/v). Quantification was carried out at 280 nm. Separation was performed at room temperature. Flow rate was fixed at 1 mL min^{-1}. Spectral data were obtained with a photodiode array detector. All data were treated with an Agilent Chemstation and related software. The FG concentrations were determined using an external calibration method. Hesperidin (HES) and narirutin (NAT)

Table 1. Carotenoids Content (Milligrams per Milliliter) of Citrus Juices[a]

Citrus species and varieties	phytoene	phytofluene	violaxanthin	lutein	zeaxanthin	β-cryptoxanthin	β-carotene	lycopene	vitamin A value[b]
Salustiana	0.28a ± 0.03	0.17a ± 0.01	4.76a ± 0.51	0.60ab ± 0.05	1.2a ± 0.05	1.50ab ± 0.11	0.11a ± 0.01	nd[c]	141 ± 11
Hamlin	0.26a ± 0.03	0.25ab ± 0.02	5.90b ± 0.82	0.86b ± 0.08	1.51a ± 0.09	1.35b ± 0.07	0.12a ± 0.01	nd	132 ± 7.0
Maltaise	0.37b ± 0.02	0.31b ± 0.02	5.83b ± 0.70	0.72b ± 0.05	1.87ae ± 011	1.80a ± 0.30	0.14a ± 0.01	nd	173 ± 23
Shamouti	0.8d ± 0.06	0.76d ± 0.06	5.81b ± 0.56	0.50a ± 0.02	1.34a ± 0.08	2.76c ± 0.15	0.25b ± 0.04	nd	272 ± 20
Sanguinelli	0.85d ± 0.06	0.87d ± 0.07	6.97b ± 0.60	1.58c ± 0.14	3.61b ± 0.15	3.88d ± 0.24	0.36c ± 0.03	nd	381 ± 25
Valencia	0.33b ± 0.02	0.35b ± 0.02	5.18a ± 0.61	1.08c ± 0.12	2.34c ± 0.09	1.65a ± 0.07	0.30c ± 0.02	nd	188 ± 6.0
Pera	0.68cd ± 0.05	0.59c ± 0.04	4.67a ± 0.35	0.87b ± 0.17	2.46c ± 0.14	3.67d ± 0.30	0.45dc ± 0.04	nd	374 ± 29
Cara-cara	4.04e ± 0.31	11.43 ± 0.71	4.65a ± 0.20	0.96b ± 0.09	1.62ae ± 0.12	1.51ab ± 0.15	0.96e ± 0.12	1.83 ± 0.09	283 ± 29
Mandarin	0.47bc ± 0.03	0.58c ± 0.04	1.39c ± 0.07	0.41d ± 0.01	0.77f ± 0.04	10.7e ± 0.45	1.60f ± 0.15	nd	1154 ± 49
Clementine	0.59c ± 0.04	0.56c ± 0.04	2.79d ± 0.16	0.31d ± 0.01	1.03g ± 0.06	8.63f ± 0.35	1.45f ± 0.08	nd	960 ± 19

[a] Values are the mean of four independent determinations plus/minus standard deviation. Different letters in the same column indicate significant differences for β-cryptoxanthin content ($\alpha = 0.05\%$). [b] Expressed as retinol equivalents L^{-1}. [c] Not detected.

standards were diluted in DMF/water (2:1, v/v) to give 102 and 79 mg L^{-1}, respectively.

Determination of Vitamin C. Ascorbic acid and total vitamin C (ascorbic acid and dehydroascorbic acid) were determined by HPLC. The procedure used was the reduction of dehydroascorbic acid to ascorbic acid, using DL-dithiothreitol (DTT) as reducing reagent, according to a similar procedure described by Cortes Sanchez-Mata et al. (*20*). Orange juice (1 mL) was homogenized with 9 mL of extraction solution (4.5% metaphosphoric acid solution in distilled water). The resulting mixture was centrifuged, the supernatant was filtered through a 0.45 μm membrane, and duplicates of 20 μL of each extract were analyzed by HPLC. The filtrate (1 mL) was added to 0.2 mL of a solution of DTT (20 mg mL^{-1}) for 2 h at room temperature in darkness; duplicates of 20 μL of each extract were analyzed by HPLC. Results were expressed as milligrams per liter of ascorbic acid. Separation of ascorbic acid was performed by HPLC (Agilent model 1100 system) using an RP 18e Licrospher 100 (5 μm) column (250 mm × 4.6 mm i.d.) (Merck KgaA). The solvent system used was an isocratic gradient of a 0.01% solution of H_2SO_4 adjusted to pH 2.5 (*21*). The flow rate was fixed at 1 mL min^{-1}. The UV−visible photodiode array detector was set at 245 nm. Quantification of ascorbic acid was performed at 245 nm by external standard calibration. Calibration curves were performed with four concentrations of ascorbic acid (100 to 30 mg L^{-1}). Straight-line equations and their coefficients of correlation were calculated ($r = 0.999$).

RESULTS

Carotenoid Content. The main nutritional carotenoids characterized in the saponified extracts from orange, Mandarin, and Clementine juices were lutein, zeaxanthin, β-cryptoxanthin, β-carotene, and lycopene (for only the Cara-cara variety). Besides these compounds, the major carotenoid of these citrus juices was violaxanthin, a yellow xanthophyll. The two precursors phytoene and phytofluene absorbing at 290 and 350 nm were also determined. Results are reported in **Table 1**. In most varieties, β-cryptoxanthin was the most abundant nutritional carotenoid. The range for orange juice was from 1.35 mg L^{-1} in cv. Hamlin to 3.88 mg L^{-1} in cv. Pera. Mandarin and Clementine juices were especially rich in β-cryptoxanthin compared to orange juice, with 10.7 and 8.63 mg L^{-1}, respectively. The carotenoid content of Mandarin was in agreement with that of several authors from Asiatic countries, where β-cryptoxanthin was the main provitamin A carotenoid because of the frequency of Mandarin consumption (*10*, *22*, *23*). Among the orange varieties, Pera, Sanguinelli, and Shamouti displayed the highest contents of β-cryptoxanthin (3.67, 3.88, and 2.76 mg L^{-1}, respectively). Cv. Sanguinelli, which is a typical Mediterranean variety with hydrosoluble red anthocyanin pigments (*24*), had the highest content of β-cryptoxanthin. In Mandarin and Clementine, β-cryptoxanthin represents the major carotenoid, with 10.7 and 8.63 mg L^{-1}, respectively. In addition, it was shown that the hybrid between Mandarin and orange (Clementine) displayed a high content of β-cryptoxanthin, similar to the Mandarin parent as described by Goodner et al. (*25*).

Table 2. Flavanone Glycoside Content (Milligrams per Liter) of Citrus Juices[a]

Citrus species and varieties	hesperidin	narirutin	ratio hesperidin/ narirutin
Salustiana	373 ± 1	53.3 ± 0.3	7.0
Hamlin	317 ± 5	56.0 ± 0.3	5.6
Maltaise	400 ± 1	96.0 ± 0.8	4.2
Shamouti	552 ± 3	94.2 ± 0.2	5.8
Sanguinelli	537 ± 6	75.3 ± 0.6	7.1
Valencia	257 ± 3	51.4 ± 0.4	5.0
Pera	502 ± 3	86.2 ± 1.0	5.8
Cara-cara	363 ± 4	98.4 ± 0.3	3.7
Mandarin	767 ± 9	37.2 ± 2.2	20.6
Clementine	754 ± 1	46.4 ± 1.6	16.2

[a] Mean of three extractions ± standard deviation.

Table 3. Vitamin C Content of Citrus Juice (Milligrams per 100 mL)[a]

Citrus species and varieties	ascorbic acid	total vitamin C
Salustiana	56.5 ± 1.2	56.8 ± 1.1
Hamlin	62.0 ± 1.3	62.7 ± 1.4
Maltaise	57.5 ± 1.3	58.2 ± 1.0
Shamouti	45.8 ± 0.9	46.2 ± 0.6
Sanguinelli	53.5 ± 0.9	53.9 ± 1.1
Valencia	55.1 ± 0.8	55.4 ± 1.0
Pera	58.8 ± 1.0	59.9 ± 1.2
Cara-cara	48.8 ± 0.9	49.1 ± 0.8
Mandarin	40.0 ± 0.8	41.3 ± 1.0
Clementine	52.8 ± 0.9	53.1 ± 0.9

[a] Mean of three extractions ± standard deviation.

Flavanone Glycoside Content. There are several flavanone glycosides specific to *Citrus*. Flavanones in orange juices were previously used as markers to differentiate citrus varieties (*26*, *27*). The main flavanone glycosides identified in orange juices and in Mandarin species were hesperidin and narirutin. Concentrations of these flavanone glycosides for all of the varieties and species are reported in **Table 2**. Hesperidin and narirutin contents for orange varieties vary from 397 to 552 mg L^{-1} and from 51.4 to 98.4 mg L^{-1}, respectively. Mandarin and Clementine displayed the highest concentrations in hesperidin (767 and 754 mg L^{-1}, respectively). Among the orange varieties, Shamouti, Sanguinelli, and Pera had the highest hesperidin contents (552, 537, 502 mg L^{-1}, respectively) and Valencia the lowest (257 mg L^{-1}). Lower hesperidin contents were observed in previous studies (*28*, *29*) for Pera and Valencia varieties. This

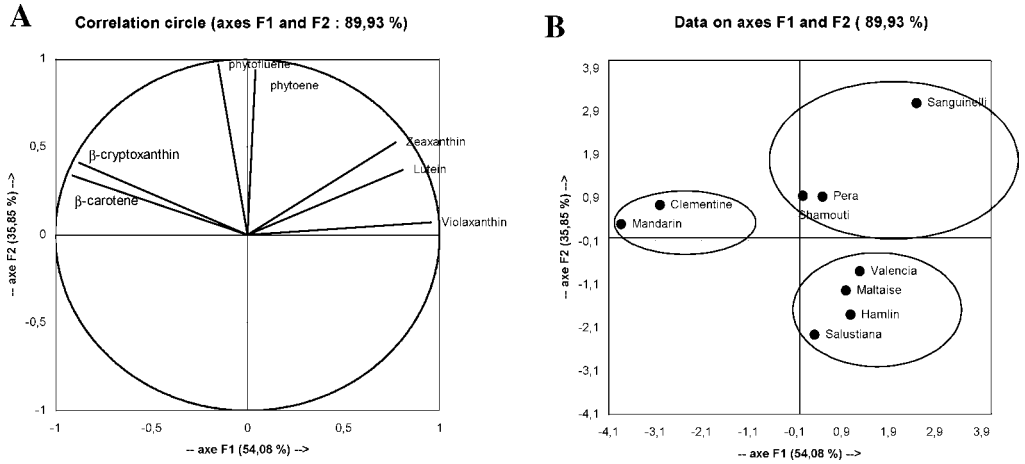

Figure 1. (**A**) Results of applying PCA to the data and (**B**) differentiation using PCA of citrus varieties.

Table 4. Correlation Matrix in PCA

	violaxanthin	lutein	zeaxanthin	β-cryptoxanthin	β-carotene	phytofluene	phytoene
violaxanthin	1	0.705	0.686	–0.847	–0.884	–0.028	0.163
lutein	0.705	1	0.946	–0.452	–0.516	0.161	0.228
zeaxanthin	0.686	0.946	1	–0.394	–0.459	0.324	0.424
β-cryptoxanthin	–0.847	–0.452	–0.394	1	0.986	0.487	0.265
β-carotene	–0.884	–0.516	–0.459	0.986	1	0.429	0.194
phytofluene	–0.028	0.161	0.324	0.487	0.429	1	0.952
phytoene	0.163	0.228	0.424	0.265	0.194	0.952	1

could be due either to the origin of fruits (tropical or subtropical) or to analytical and extraction procedures.

Vitamin C Content. Citrus are well-known to be a nutrient source of vitamin C in dietary intake. Data reported in **Table 3** give both ascorbic acid and total vitamin C values. No significant differences were found between ascorbic acid and total vitamin C. Dehydroascorbic acid concentrations were quite low and were at the limit of detection in our study. The range for ascorbic acid content was from 40 to 62 mg 100 mL^{-1} for all of the citrus juices. The Mandarin/Clementine group had slightly lower values (40 and 52.8 mg 100 mL^{-1}, respectively), whereas the orange group had a mean of 54.7 mg 100 mL^{-1}.

Principal component analysis (PCA) was used to analyze the relationships between the concentrations of five carotenoids (violaxanthin, lutein, zeaxanthin, β-cryptoxanthin, and β-carotene) and the two colorless precursors (phytoene and phytofluene) among the nine cultivars analyzed (Cara-cara was excluded because of its very particular composition). Results are reported in **Figure 1** and **Table 4**. Three strong correlations were obtained between β-cryptoxanthin and β-carotene ($r = 0.98$), between phytoene and phytofluene ($r = 0.95$), and between zeaxanthin and lutein ($r = 0.94$). The first correlation can be explained by the biosynthetic pathway, where β-cryptoxanthin is formed by the hydroxylation of β-carotene. These two carotenoids were very particularly linked in citrus varieties in which β-cryptoxanthin was present at a high level. It is known that in orange and Mandarin, the principal event occurring during carotenogenesis is the increase of β-cryptoxanthin with maturity (*30*, *31*). The other correlations reflect the biosynthesis pathways of carotenoids.

The same multivariate analysis (**Figure 1B**) allowed the nine citrus cultivars to be classified (Salustiana, Hamlin, Maltaise, Shamouti/Jaffa, Sanguinelli, Valencia, Pera, Mandarin, and Clementine) into different categories based on nutritional criteria, especially the carotenoid content. The Mandarin/Clementine group was clearly differentiated from sweet oranges, and this

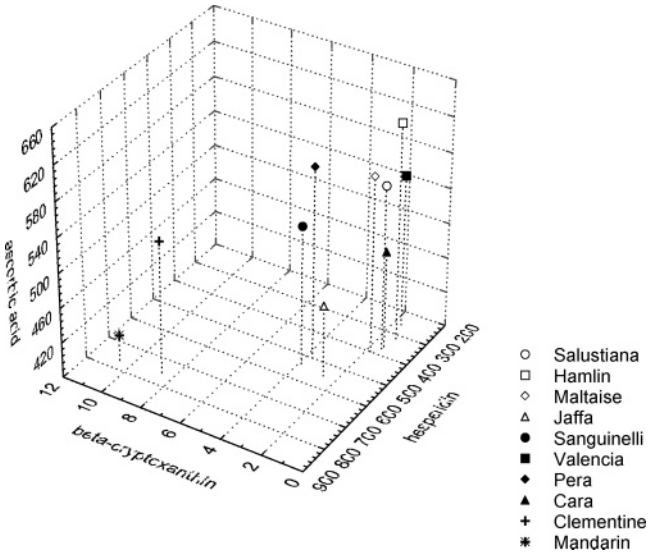

Figure 2. Differentiation of citrus varieties and species from Mediterranean origin based on three principal micronutrients: β-cryptoxanthin (mg L^{-1}), hesperidin (mg L^{-1}), and ascorbic acid (mg L^{-1}). Representation was made with Statistica software.

group was highly correlated with β-cryptoxanthin and β-carotene contents. A second group consisted of four varieties of orange juices that had low contents of β-cryptoxanthin and β-carotene; this group was more correlated with lutein and zeaxanthin contents. The last three varieties of orange could be distinguished by their higher contents of β-cryptoxanthin and β-carotene.

DISCUSSION

β-Cryptoxanthin, mainly present as fatty acid esters, was shown to be the major provitamin A carotenoid in *Citrus*. Its content was found to be particularly high in Mandarin and Clementine. To get the classification of Mediterranean *Citrus*

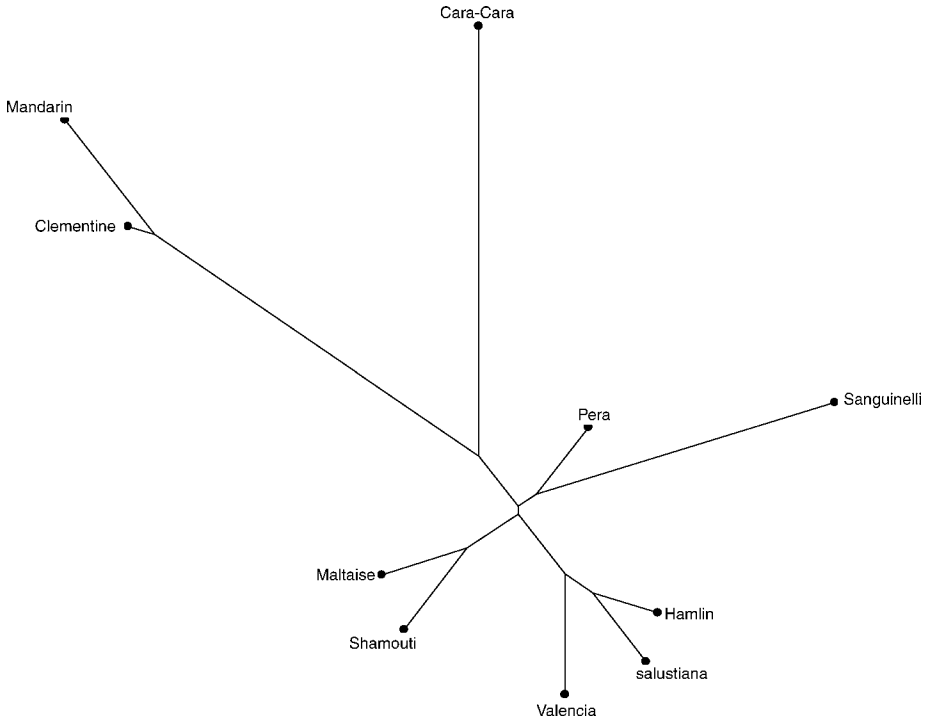

Figure 3. Classification of sweet oranges, Clementine, and Commune Mandarin on the basis of nutritional components. Neighbor-joining analysis on Euclidian distances from carotenoids and flavonoids contents was performed.

cultivars based on nutritional criteria, correlations between β-cryptoxanthin and other antioxidants, hesperidin and vitamin C, were examined. A strong correlation was observed between β-cryptoxanthin and hesperidin ($r = 0.927$), which stressed that some cultivars have high contents of both of these antioxidants. Such a correlation between these two antioxidants was surprising, because of their independent biosynthetic pathways. Even though these two antioxidants (β-cryptoxanthin and hesperidin) were biosynthetically independent, it may be possible that their accumulation was governed by the same factors during fruit ripening. A three-dimensional representation of the results (**Figure 2**) clearly showed the most interesting cultivars with regard to their nutritional qualities. The Mandarin/Clementine group was the most interesting with respect to their hesperidin and β-cryptoxanthin contents. It is quite remarkable to note that Clementine, which is known to be a hybrid between the Mediterranean Mandarin (*Citrus deliciosa*) and a sweet orange, is very close to its Mandarin parent with regard to its nutritional characteristics (**Figure 3**). These results confirm that "Mediterranean Mandarin" exerts a strong dominance for the expression of secondary metabolites as has previously been observed for aromatic compounds of somatic hybrids by Gancel et al. (*32*). With regard to its high nutritional value and genetic dominance, Mediterranean Mandarin appeared as a very interesting parent for citrus breeding with a nutritional objective.

Among the sweet oranges, Shamouti, Sanguinelli, and Pera displayed the best combination of hesperidin and β-cryptoxanthin, with a good level of vitamin C for the last two cultivars. Salustiana, Hamlin, Maltaise, and Valencia sweet oranges appeared to be less interesting from a nutritional point of view. Among the most interesting sweet oranges, cv. Pera is especially produced in Brazil and widely distributed over the world. Compared to the well-known Valencia variety that is widely processed into juices, the Pera variety had better nutritional characteristics, particularly in vitamin A (374 RE) and hesperidin contents. Cv. Sanguinelli, which is mostly cultivated in Italy, also possessed strong nutritional qualities, with the highest provitamin A value as well as high contents of hesperidin and vitamin C. This suggests that this cultivar should be promoted for both the fresh market and juice processing.

Concerning cv. Cara-Cara, this unique red navel orange was shown to contain the red lipophilic pigment lycopene as in red grapefruit (1.83 mg L^{-1}), as well as a high content of β-carotene (2 to 9 times more than other sweet oranges). Besides the provitamin A activity provided by β-carotene and β-cryptoxanthin, lycopene could contribute to the high antioxidant capacity of cv. Cara-cara. Indeed, lycopene has been shown to possess a high antioxidant function among carotenoids because of its highly conjugated structure (*33*).

Finally, this study confirmed the high contents of carotenoids in citrus varieties cultivated under the Mediterranean climate. Indeed, climatic conditions in Corsica during the 2002 season were similar to the those of the past 20 years, with low average temperatures (15 °C). Under these more stressful conditions, the citrus skin and pulp color appeared to be more pronounced than those in citrus fruits produced under tropical or subtropical climates, reflecting a higher content of carotenoids in Mediterranean citrus fruits. To confirm the higher content in antioxidants of citrus growing under the Mediterranean climate, studies are currently being carried out for the same varieties cultivated under both Mediterranean and tropical climates.

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LITERATURE CITED

(1) Benavente-Garcia, O.; Castillo, J.; Marin, F. R.; Ortuno, A.; Del Rio, J. A. Uses and properties of citrus flavonoids. *J. Agric. Food Chem.* **1997**, *45*, 4505−4515.

(2) Kawaii, S.; Tomono, Y.; Katase, E.; Ogawa, K.; Yano, M. HL-60 differentiating activity and flavonoid content of the readily extractable fraction prepared from *Citrus* juice. *J. Agric. Food Chem*. **1999**, *47*, 128−135.
(3) Yano, M. Cancer prevention by citrus. *J. Jpn. Soc. Food Sci. Technol.* **2002**, *49*, 139−144.
(4) Garner, P. T.; White, T. A. C.; McPhail, D. B.; Duthie, G. G. The relative contributions of vitamin C, Carotenoids and phenolics to the antioxidant potential of fruit juices. *Food Chem.* **2000**, *68*, 471−474.
(5) Sanchez-Moreno, C.; Plaza, L.; De Ancos, B.; Cano, P. Quantitative bioactive compounds assessment and their relative contribution to the antioxidant capacity of commercial orange juices. *J. Sci. Food Agric*. **2003**, *83*, 430−439.
(6) Rapisarda, P.; Tomaino, A.; Lo Cascio, R.; Bonina, F.; De Pasquale, A.; Saija, A. Antioxidant effectiveness as influenced by phenolic content of fresh orange juices. *J. Agric. Food Chem.* **1999**, *47*, 4718−4723.
(7) Erlund, I.; Meririnne, E.; Alfthan, G.; Aro, A. Plasma kinetics and urinary excretion of the flavanones naringenin and hesperetin in humans after ingestion of orange juice and grapefruit juice. *J. Nutr*. **2001**, *131*, 235−241.
(8) Manach, C.; Morand, C.; Gil-Iquierdo, A.; Bouteloup-Demange, C.; Rémésy, C. Bioavailability in humans of the flavanones hesperidin and narirutin after the ingestion of two doses of orange juice. *Eur. J. Clin. Nutr*. **2002**, *57*, 235−242.
(9) Nagy, S.; Chen, C. S.; Shaw, P. E. *Fruit Juice Processing Technology*; Agscience: Auburndale, FL, 1993.
(10) Lin, S. D.; Chen, A. O.; Major carotenoids in juices of ponkan mandarin and Liucheng orange. *J. Food Biochem*. **1995**, *18*, 273−283.
(11) Miller, N. J.; Sampson, J.; Candeias, L. P.; Bramley, P. M.; Rice-Evans, C. A. Antioxidant activities of carotenes and xanthophylls. *FEBS Lett.* **1996**, *384*, 240−242.
(12) Sugiura, M.; Kato, M.; Matsumoto, H.; Nagao, A.; Yano, M. Serum concentration of β-cryptoxanthin in Japan reflects the frequency of satsuma mandarin (*Citrus unshiu*. Marc.) consumption. *J. Health Sci.* **2002**, *48*, 350−353.
(13) Irwig, M. S.; El-Sohemy, A.; Baylin A.; Rifai, N.; Campos, H. Frequent intake of tropical fruits that are rich in β-cryptoxanthin is associated with higher plasma β-cryptoxanthin concentrations in Costa Rican adolescents. *J. Nutr*. **2002**, *132*, 3161−3167.
(14) Gil-Izquierdo, A.; Gil, I. M.; Ferreres, F. Effect of processing techniques at industrial scale on orange juice antioxidant and beneficial health compounds. *J. Agric. Food Chem*. **2002**, *50*, 5107−5114.
(15) Pupin, A. M.; Dennis, M. J.; Toledo, M. C. F. HPLC Analysis of carotenoids in orange juice. *Food Chem*. **1999**, *64*, 269−27514. Mouly, P. P.; Gaydou, E. M.; Faure, R.; Estienne, M. Blood orange juice authentication using cinnamic acid derivatives. Variety differentiations associated with flavanone glycoside content. *J. Agric. Food Chem.* **1997**, *45*, 373−377.
(16) Taugbodhitham, A. K.; Jones, G. P.; Walhlqvist, M. L.; Briggs, D. R. Evaluation of method for the analysis of carotenoids in fruits and vegetables. *Food Chem*. **1998**, *63*, 577−584.
(17) Caris-Veyrat, C.; Schmid, A.; Carail, M.; Böhm, V. Cleavage products of lycopene produced by in vitro oxidations: characterization and mechanisms of formation. *J. Agric. Food Chem* **2003**, *51*, 7318−7325.
(18) Britton, G.; Liaanen-Jensen, S.; Pfander, H. *Carotenoids. Vol. 1B: Spectroscopy*; Birkhaüser: Basel, Switzerland, 1995.
(19) Mouly, P. P.; Gaydou, E. M.; Faure, R.; Estienne, M. Blood orange juice authentication using cinnamic acid derivatives. Variety differentiations associated with flavanone glycoside content. *J. Agric. Food Chem.* **1997**, *45*, 373−377.
(20) Cortes Sanchez-Mata, M.; Camara-Hurtando, M.; Diez-Marques, C.; Esperanza Torija-Isasa, M. Comparaison of high-performance liquid chromatography and spectrofluorimetry for vitamin C analysis of green beans (*Phaseolus vulgaris* L.). *Eur. Food Res. Technol.* **2000**, *210*, 220−225.
(21) Sanchez-Moreno, C.; Plaza, L.; De Ancos, B.; Cano, P. Vitamin C, provitamin A, and other carotenoids in high-pressurized orange juice during refrigerated storage. *J. Agric. Food Chem.* **2003**, *51*, 647−653.
(22) Sumida, T.; Hamada, S.; Azuma, Y.; Ogawa, H.; Tada, M. Change of total carotenoid and β-cryptoxanthin contents of satsuma mandarin (*Citrus unshiu* Marc.) juice caused by heating, illumination and storage. *Nippon Shokulin Kagaku Gakkaishi* **1999**, *6*, 106−110.
(23) Kiong-Cheol Ko; Chan-Shick Kim; Nam Ho Lee; Sam-Pin Lee; Doo-Khil Moon. Determination of β-cryptoxanthin in peel and flesh of citrus fruits produced in Cheju Island. *Food Sci. Biotechnol*. **2000**, *9*, 288−291.
(24) Saunt, J. *Citrus Varieties of the World*; Sinclair International: Norwich, U.K., 2000.
(25) Goodner, K. L.; Rouseff, R. L.; Hofsommer, H. J. Orange, mandarin, and hybrid classification using multivariate statistics based on carotenoid profiles. *J. Agric. Food Chem.* **2001**, *49*, 1146−1150.
(26) 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.
(27) Ooghe, W. C.; Ooghe, S. J.; Detavernier, C. M.; Huyghebaert, A.; Characterization of orange juice (C*itrus sinensis*) by flavanone glycosides. *J. Agric. Food Chem.* **1994**, *42*, 2183−2190.
(28) Rouseff, R. L.; Martin, S. F.; Youtsey, C. O. Quantitative survey of narirutin, naringin, hesperidin and neohesperidin in *Citrus*. *J. Agric. Food Chem.* **1987**, *35*, 1027−1030.
(29) Pupin, A. M.; Dennis, M. J.; Toledo, M. C. F. Flavanones glycosides in brazilian orange juice. *Food Chem.* **1998**, *61*, 275−280.
(30) Gross, J. Carotenoid changes in the juice of the ripening Dancy tangerine (*Citrus reticulata*). *Lebensm.-Wiss - Technol.* **1982**, *15*, 36−38.
(31) Lee, H. S.; Castle, W. S. Seasonal changes of carotenoid pigments and color in Hamlin, Earlygold, and Budd Blood Orange juices. *J. Agric. Food Chem.* **2001**, *49*, 877−882.
(32) Gancel, A. L.; Ollitrault, P.; Froelicher, Y.; Tomi, F.; Jacquemond, C.; Luro, F.; Brillouet, J. M. Leaf volatile compounds of seven citrus somatic tetraploid hybrids sharing willow leaf mandarin (*Citrus deliciosa* Ten.) as their common parents. *J. Agric. Food Chem*. **2003**, *51*, 6006−6013.
(33) Di Mascio, P.; Murphy, M. C.; Sies, H. Antioxidant defense systems, the role of carotenoid, tochopherol and thiols. *Am. J. Clin. Nutr. Suppl.* **1991**, *53*, 194−200.

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