Eur Food Res Technol (2007) 225:271–278
DOI 10.1007/s00217-006-0414-x

ORIGINAL PAPER

Total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities of juices from clementine (*Citrus clementina*), clementine-hybrids and satsuma (*Citrus unshiu*) cultivars and their utility as discriminant variables

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Received: 29 March 2006 / Revised: 9 June 2006 / Accepted: 16 June 2006 / Published online: 15 August 2006

Abstract Mandarin juices from three pure clementine (*Citrus clementina* Hort. ex Tan.) cultivars: Clemenules, Hernandine and Marisol, two clementine-hybrid cultivars: Nova (clementine × tangelo Orlando) and Fortune (clementine × mandarin Dancy), one mandarin-hybrid cultivar: Ortanique (mandarin × orange) and one satsuma (*Citrus unshiu* Marc) cultivar: Clauselline have been analysed for determination of total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities. Total antiradical activity followed the order: pure clementine and Nova> Ortanique>Fortune and Clauselline. Cumulative fast-kinetics antiradical activity was the major contributor to total antiradical activity, accounting for about 87% as average. Ascorbic acid content was the major contributor to cumulative fast-kinetics antiradical activity, accounting for about 93.5% as average. A weak correlation ($r^2 = 0.151$) between total flavanone-7-*O*-glycosides (FGs, Narirutin, Hesperidin and Didymin) content and cumulative slow-kinetics antiradical activity was observed. However, ascorbic acid and total FGs contents showed a significant negative correlation ($r^2 = 0.658$). By using the cumulative fast-kinetics and slow-kinetics antiradical activities as variables, all the assayed juices were significantly discriminated (<0.05) by statistical multivariate discriminant analysis.

Keywords Antiradical activity · Mandarin juices · *Citrus clementina* · *Citrus unshiu* · Clementine-hybrids · Characterization · Multivariate discriminant analysis

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Introduction

It seems that the beneficial effects on human health due to the consumption of citrus are known for a long time, since this consumption was already recommended in the first book on citrus plants written by a Chinese writer in 1178 during the Sung dynasty [1]. Nowadays, we know that these beneficial effects are mainly due to the antioxidant and antiradical properties of citrus fruit components and, particularly, of those found in their juices. Citrus juices are well supplied with substances exhibiting antioxidant and antiradical activities, such as ascorbic acid [2], flavonoids [3], carotenoids [4], anthocyanins (only in juices from pigmented citrus varieties) [5, 6], cinnamic acid derivatives [7], etc. Citrus juice intake affects both Vitamin C concentration and biomarkers of antioxidant status in human blood [8], thus being bioactive against a series of human illnesses such as coronary heart disease [9] and some age-related disorders [10], as well as preventive against the risk of developing certain types of cancer [11–13].

A review of published methodologies for the determination of the antioxidant and antiradical activity of isolated compounds, foods, juices and biological systems [14], as well as a review about the chemistry behind antioxidant capacity assays [15] have been published recently.

There is abundant scientific bibliography dealing with the determination of total antiradical activity of citrus juices, mainly from sweet orange, as well as with the partial contributions of the different groups of citrus juice components to this activity. Miller and Rice-Evans [16] analysed several sweet orange juices for antiradical activity, using the ferrylmyoglobin/ABTS assay, and concluded that ascorbic acid accounts for about 87% of the total antiradical activity. Gardner et al. [17] used spin electron resonance to determine the ability of Vitamin C, total phenols and total

carotenoids from several orange juices to reduce the synthetic free radical potassium nitrosodisulphonate (Fremy's salt) as well as Fe(III)-2,4,6-tri(2-pycryl)-s-triazine (TPTZ) to Fe(II)-TPTZ. Results from both assays were very similar and the relative contribution of Vitamin C to the total antioxidant activity was estimated to be from 65 to 100%. Sanchez-Moreno et al. [18], using the DPPH$^{\bullet}$ assay, determined the relative contributions of Vitamin C, flavonoids and carotenoids to the total antiradical activity of several sweet orange juices, concluding that Vitamin C is by far the major contributor (>95%) to this activity. Pretel et al. [19], using the ABTS/peroxidase/H_2O_2 assay, determined the total antiradical activity of the edible tissues (juice, albedo and carpelar membranes) of orange fruits from 17 non-pigmented orange varieties, concluding that the relative contribution of ascorbic acid to the total antiradical activity of the juices was 50–82%. Rapisarda et al. [20] determined the antiradical activity of several juices from pigmented orange varieties, using the DPPH$^{\bullet}$, LP-LUV and NO (nitric oxide) assays, and reported that due to their high content in anthocyanins, the relative contribution of total phenols to the total antiradical activity was greater by far than that of ascorbic acid, which accounted for about 15%.

Very recently, Sendra et al. [21] published a theoretical approach to the reduction kinetics of DPPH$^{\bullet}$ by antiradicals found in citrus juices. These antiradicals were grouped, according to their reaction kinetic with DPPH$^{\bullet}$, into three general groups: fast-kinetics, fast + slow-kinetics and slow-kinetics. Hence, the total antiradical activity of a citrus juice is the sum of its cumulative fast-kinetics and cumulative slow-kinetics antiradical activities. Moreover, a kinetic equation for fitting both the experimental data points from isolated compounds belonging to the fast-kinetics or to the fast + slow-kinetics group and from citrus juices was deduced, thus allowing the determination of total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities of citrus juices.

There are almost no scientific references dealing with the determination of the antiradical activity of mandarin juices [22]. Hence, the goal of this work is to determine the total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities, as well as the ascorbic and dehydroascorbic acid contents, of juices from three pure clementine (*Citrus clementina* Hort. ex Tan.) cultivars: Clemenules, Marisol and Hernandine; two clementine-hybrid cultivars: Nova (clementine × tangelo Orlando) and Fortune (clementine × mandarin Dancy); one mandarin-hybrid cultivar: Ortanique (mandarin × orange); and one satsuma (*Citrus unshiu* Marc) cultivar: Clauselline. The determined values of total, cumulative fast-kinetics and cumulative slow-kinetics antiradical activities were almost specific of each assayed cultivar. Cumulative fast-kinetics antiradical activity was the major contributor to total antiradical activity; ascorbic acid (or Vitamin C) content was the major contributor to cumulative fast-kinetics antiradical activity; total flavanone-7-*O*-glycosides (FGs, Hesperidin, Narirutin and Didymin) content of juices was almost uncorrelated with their cumulative slow-kinetics antiradical activity, but a rather significant negative correlation between ascorbic acid and total FGs contents was observed. Finally, statistical multivariate discriminant analysis showed that by using cumulative fast-kinetics and slow-kinetics antiradical activities as variables, all assayed mandarin juices were significantly (<0.05) distinguished.

Materials and methods

Assayed cultivars

Distinct fruit samples (about 50 kg) of each cultivar were randomly collected from different orchards, located in the Comunidad Valenciana (Spain). Fruits from each sample were hand-squeezed and the resulting juice was either immediately analysed or stored at -20 °C until analysis, which was carried out within the next 2 days. Data on the assayed cultivars are given in Table 1.

Reagents and standards

Methanol (spectrophotometric grade), dithiothreitol (DTT), ascorbic acid and DPPH$^{\bullet}$ (95% purity) were from Sigma (Sigma Aldrich Co., St. Louis, MO, USA). HPLC-grade methanol and acetonitrile were obtained from Panreac (Panreac Química S.A., Barcelona, Spain). Water used was Milli-Q grade (Millipore Ibérica, Spain). C-18 Sep-Pak cartridges were obtained from Waters (Waters Co., Milford, MA, USA).

Vitamin C determination in citrus juices

Citrus juice preparation

Sample preparation was carried out following published methodologies [23, 24] with minor modifications. Twenty millilitres of juice were centrifuged at 13 000 rpm for 5 min at room temperature, the supernatant was decanted and filtered through a 0.45 μm membrane filter. The filtered juice was passed through a C-18 Sep-Pack cartridge, previously activated by passing 5 mL of acetonitrile and washing with 5 mL of water. The first 5 mL of eluate were discarded and the next 6 mL were collected. From the collected eluate, two aliquots of 2.5 mL were prepared, the former for determining ascorbic acid and the latter for determining Vitamin C (ascorbic plus dehydroascorbic acids). The first aliquot, after addition of 0.5 mL of water, was filtered through a 0.45 μm

Table 1 Harvest dates and maturity indexes of the assayed mandarin and mandarin-hybrid cultivars

Cultivar	Species	Code	Harvest date	Brix	Maturity index (Brix/acid)
Clauselline	Satsuma (*Citrus unshiu* Marc.)	CLS-1	15/10/2003	9.3	11.20
		CLS-2	30/10/2003	9.5	12.00
Marisol	Clementine (*Citrus clementina* Hort. ex Tan.)	MRS-1	30/10/2003	10.7	8.70
		MRS-2	19/11/2003	10.8	11.13
		MRS-3	1/12/2003	11.8	13.26
Clemenules	Clementine (*Citrus clementina*)	CMN-1	30/10/2003	10.6	9.64
		CMN-2	10/12/2003	11.0	16.40
		CMN-3	15/01/2004	12.0	22.20
Hernandine	Clementine (*Citrus clementina*)	HRN-1	10/12/2003	11.5	13.85
		HRN-2	8/01/2004	12.4	20.3
		HRN-3	20/01/2004	14.1	24.70
Nova	Hybrid between Clementine (*Citrus clementina*) × tangelo Orlando (*Citrus paradisi* Macf. × *Citrus tangerina* Hort. ex Tan.)	NOV-1	10/12/2003	11.5	12.50
		NOV-2	8/01/2004	12.2	19.10
		NOV-3	20/01/2004	13.2	18.30
Ortanique	Hybrid between mandarin (*Citrus reticulata* Blanco) × Orange [*Citrus sinensis* (L.) Osb.]	ORT-1	12/02/2004	13.8	9.70
		ORT-2	25/02/2004	13.6	10.15
		ORT-3	6/04/2004	12.4	10.51
		ORT-4	29/04/2004	12.7	13.50
Fortune	Hybrid between Clementine (*Citrus clementina*) × mandarin Dancy (*Citrus tangerina*)	FRT-1	25/02/2004	15.4	9.11
		FRT-2	6/04/2004	16.6	10.64

membrane filter and poured into a 2 mL septum-capped glass vial for HPLC determination of ascorbic acid concentration. The second aliquot, after addition of 0.5 mL of DTT (20 g L^{-1}), was left to stand for 2 h in darkness to convert dehydroascorbic acid into ascorbic acid. The mixture was then filtered through a 0.45 μm membrane filter and poured into a 2 mL septum-capped glass vial for HPLC determination of Vitamin C. The total amount of dehydroascorbic acid was calculated as the difference of ascorbic acid contents between both aliquots. All samples were analysed in duplicate.

HPLC analysis

Ascorbic acid in citrus juices was quantified using a high performance liquid chromatograph, Agilent model 1100, equipped with vacuum degasser, binary pump, automatic injector, autosampler and diode array detector. The instrument was controlled and the chromatographic data were analysed using a personal computer loaded with the HP ChemStation software. The column used was a 5 μm reverse-phase C-18, 25 cm length and 4.6 mm i.d. (Luna II, Phenomenex, Torrance, USA). Operating conditions were: detection wavelength, 245 nm; mobile phase, MeOH/H_2O (5:95) adjusted to pH 3.5 with H_3PO_4; flow rate, 1 mL min^{-1}; injection volume, 5 μL; and column temperature, ambient.

Prior to quantification of ascorbic acid, a calibration curve of concentration of an ascorbic acid standard (0–1000 mg mL^{-1}, step 100 mg mL^{-1}) vs. chromatographic peak area was obtained for the subsequent conversion of peak area to ascorbic acid concentration.

Antiradical activity determination

Citrus juice preparation

Ten millilitres of citrus juice were centrifuged at 13 000 rpm for 5 min at room temperature, the supernatant was decanted and filtered through Whatman filter paper #1. A volume (V_o, between 10–70 μL) of filtered juice was added to a cuvette containing an appropriate volume of DPPH$^\bullet$ in methanol to yield a final volume of 3 mL (the final concentration of DPPH$^\bullet$ was around 100 μmol L^{-1}). The cuvette was end-capped immediately, shaken by hand and placed in the spectrophotometer for analysis. Reaction time data were corrected taking into account the time at which the juice was added and the time given by the spectrophotometer. All samples were analysed in duplicate.

UV/Vis analysis

Absorbance was measured using an Amersham (Amersham Pharmacia Biotech., Cambridge, England) spectrophotometer, model 3300 *pro*, set at a wavelength of 515 nm. Spectrophotometric quartz cuvettes (3.5 mL capacity and 1 cm path-length) were used. Usually, four to six samples plus a blank were analysed simultaneously at a sampling rate of one point per 2 min per sample. Automatic acquisition of data was stopped after a reaction time of about 60–70 min. All samples were analysed in duplicate at room temperature.

Results and discussion

Antiradical activity of the assayed juices

Figure 1(A) shows, as an example, the time course evolution of the concentration (μmol L^{-1}) of DPPH$^{\bullet}$ when it is being reduced by Clemenules juice (sample CMN-1). According to Sendra et al. [21], each series of data points (curve) was fitted using the simplified global kinetic equation:

$$y - y_s = \frac{y_o - y_1}{1 + \rho_1 y_o t} + \frac{y_2(y_o - y_2)}{y_2 - y_o(1 - e^{\rho_2 y_2 t})}$$

but forcing the constraint $y_2 = y_o + y_s - y_1$, where y is the time dependent concentration of DPPH$^{\bullet}$; y_s is the final (asymptotic) concentration of DPPH$^{\bullet}$; y_o is the initial concentration of DPPH$^{\bullet}$; y_1 is the final concentration of DPPH$^{\bullet}$ that would be reached due solely to the cumulative fast-kinetics antiradical activity of the juice; y_2 is the final concentration of DPPH$^{\bullet}$ that would be reached due solely to the cumulative slow-kinetics antiradical activity of the juice; and $\rho_i = k_i/\sigma_i$ ($i = 1, 2$), being k_i and σ_i the average rate and stoichiometric constants, respectively, corresponding to the cumulative fast-kinetics ($i = 1$) and slow-kinetics ($i = 2$) antiradical activities of the juice. It must be emphasized that the lack of very early data points, and the subsequent use of the simplified kinetic equation for fitting, prevent the accurate determination of the average rate constant for cumulated fast-kinetics antiradical activity. However, this has little real importance since in mandarin juices this value is almost identical to that of ascorbic acid but the average stoichiometric constant for cumulated fast-kinetics, which is the important value for antiradical activity, can accurately be determined.

Figure 1(B) shows the graphical representation of $(y_o - y_s)$, $(y_o - y_1)$ and $(y_o - y_2) = (y_1 - y_s)$ (μmol L^{-1}) vs. V_o (μL), the volume of juice added. According to Sendra et al. [21], the slope $m_s = (y_o - y_s)/V_o$ quantifies the total concentration (μmol L^{-1}) of DPPH· reduced per μL of added juice; the slope $m_1 = (y_o - y_1)/V_o$ quantifies the concentration (μmol L^{-1}) of DPPH· reduced per μL of added juice, but due solely to the cumulative antiradical activity of its components exhibiting fast-kinetics; and the slope $m_2 = (y_o - y_2)/V_o$ quantifies the concentration (μmol L^{-1}) of DPPH$^{\bullet}$ reduced per μL of added juice, but due solely to the cumulative antiradical activity of its components exhibiting slow-kinetics. Taking into account the value of the stoichiometric constant of ascorbic acid ($\sigma = 2$) and the volume of the reaction (V, mL), the quotient m_s/σ is directly correlated with the total antiradical activity (A_t) of the juice ($A_t = Vm_s/\sigma$ mmol L^{-1}, expressed as molar equivalents of ascorbic acid); the quotient m_1/σ is directly correlated with the cumulative antiradical activity (A_1) due to the juice components exhibiting fast-kinetics ($A_1 = Vm_1/\sigma$ mmol L^{-1}, expressed as molar equivalents of ascorbic acid); and the slope m_2/σ is directly correlated with

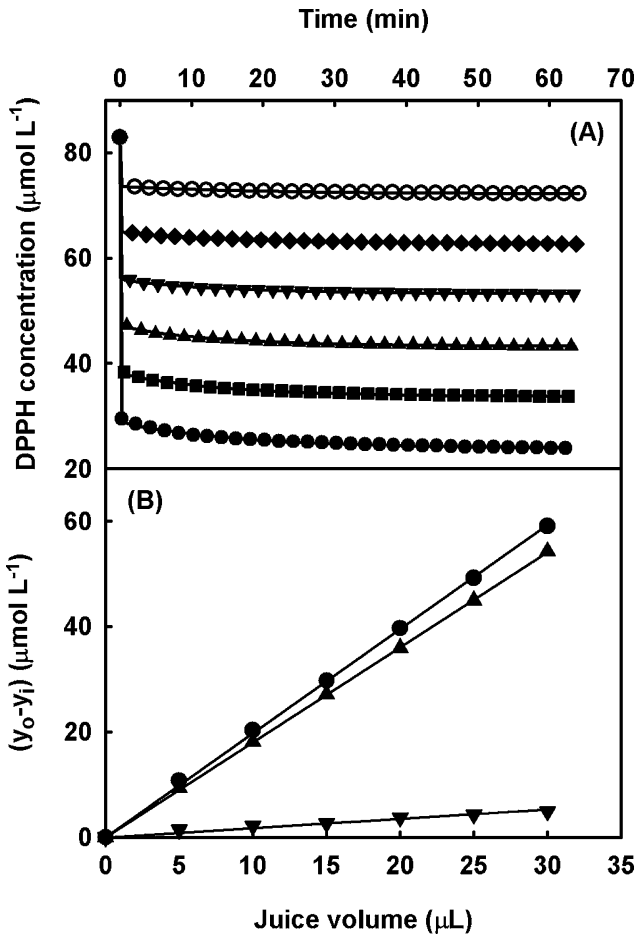

Fig. 1 (A) Time course of the reduction of the free radical DPPH$^{\bullet}$ (82.938 μmol L^{-1}) by Clemenules (sample CMN-1) juice: 30 (●), 25 (■), 20 (▲), 15 (▼), 10 (♦) and 5 (○) μL. (B) Graphical representation of the concentrations (μmol L^{-1}) of reduced DPPH$^{\bullet}$ vs. the volume of Clemenules juice (μL) added. Total concentration reduced by the juice: $y_i = y_s$ (●); concentration reduced due to cumulative fast-kinetics components: $y_i = y_1$ (▲); and concentration reduced due to cumulative slow-kinetics components: $y_i = y_2$ (▼). Correlation coefficient (r^2) > 0.998 in all cases

Table 2 Total (A_t), cumulative fast-kinetics (A_1) and cumulative slow-kinetics (A_2) antiradical activity[a] (mean ± SD), ascorbic (AA) and dehydroascorbic (DHAA) acids content[b] (mean ± SD) and total FGs (mg L^{-1}) in the juices of the assayed mandarin cultivars

Sample	A_t	A_1	A_2	AA	DHAA	Total FGs
CMN-1	2.962 ± 0.005	2.699 ± 0.006	0.262 ± 0.001	2.631 ± 0.003	<0.01	55.77
CMN-2	2.794 ± 0.013	2.550 ± 0.005	0.244 ± 0.018	2.217 ± 0.014	0.026 ± 0.004	24.64
CMN-3	2.931 ± 0.021	2.639 ± 0.013	0.292 ± 0.008	2.481 ± 0.023	<0.01	48.35
HRN-1	4.030 ± 0.090	3.750 ± 0.032	0.280 ± 0.057	3.610 ± 0.003	0.115 ± 0.001	24.59
HRN-2	4.041 ± 0.015	3.742 ± 0.012	0.299 ± 0.026	3.613 ± 0.001	0.115 ± 0.006	34.10
HRN-3	3.987 ± 0.077	3.669 ± 0.071	0.318 ± 0.007	3.353 ± 0.001	0.101 ± 0.011	24.99
MRS-1	3.123 ± 0.025	2.947 ± 0.024	0.176 ± 0.006	2.765 ± 0.009	0.030 ± 0.001	96.50
MRS-2	3.053 ± 0.036	2.865 ± 0.043	0.188 ± 0.006	2.695 ± 0.005	<0.01	73.58
MRS-3	3.262 ± 0.028	3.047 ± 0.022	0.215 ± 0.007	2.922 ± 0.013	<0.01	83.42
NOV-1	3.124 ± 0.045	2.801 ± 0.040	0.323 ± 0.005	2.587 ± 0.001	<0.01	23.67
NOV-2	3.353 ± 0.006	2.975 ± 0.008	0.378 ± 0.001	2.600 ± 0.002	0.051 ± 0.007	52.43
NOV-3	3.321 ± 0.022	2.994 ± 0.028	0.327 ± 0.006	2.724 ± 0.002	0.014 ± 0.001	81.62
FOR-1	2.095 ± 0.006	1.595 ± 0.011	0.500 ± 0.017	1.536 ± 0.006	0.034 ± 0.005	103.74
FOR-2	2.236 ± 0.003	1.733 ± 0.012	0.512 ± 0.002	1.600 ± 0.001	<0.01	117.24
ORT-1	1.870 ± 0.036	1.519 ± 0.037	0.350 ± 0.002	1.425 ± 0.003	<0.01	146.47
ORT-2	1.862 ± 0.018	1.516 ± 0.029	0.346 ± 0.010	1.426 ± 0.001	<0.01	154.84
ORT-3	1.878 ± 0.008	1.579 ± 0.012	0.299 ± 0.020	1.487 ± 0.011	<0.01	190.91
ORT-4	1.704 ± 0.007	1.431 ± 0.019	0.273 ± 0.012	1.271 ± 0.004	0.035 ± 0.002	193.98
CLS-1	1.901 ± 0.028	1.460 ± 0.027	0.448 ± 0.010	1.397 ± 0.001	0.049 ± 0.005	228.19
CLS-2	1.981 ± 0.006	1.558 ± 0.015	0.423 ± 0.013	1.514 ± 0.016	<0.01	245.33

[a]Expressed as mmol L^{-1} molar equivalents of ascorbic acid

[b]Expressed as mmol L^{-1}

the cumulative antiradical activity (A_2) due to the juice components exhibiting slow-kinetics ($A_2 = Vm_2/\sigma$ mmol L^{-1}, expressed as molar equivalents of ascorbic acid). Evidently, $A_t = A_1 + A_2$ is fulfilled. Table 2 gives the total (A_t), cumulative fast-kinetics (A_1) and cumulative slow-kinetics (A_2) antiradical activities, the concentration of ascorbic (AA) and dehydroascorbic (DHAA) acids, and the total flavanone-7-*O*-glycosides (FGs) content [25], including Narirutin, Hesperidin and Didymin, in the assayed juices.

Contributions to the total antiradical activity

Figure 2 shows the graphical representation of the total antiradical activity, A_t (mmol L^{-1} molar equivalents of ascorbic acid) vs. the cumulative fast-kinetics antiradical activity, A_1 (mmol L^{-1} molar equivalents of ascorbic acid), for all the assayed juices. Experimental data points were fitted using the straight line $A_t = mA_1 + r_t$. As it can be seen, a rather good correlation ($r^2 = 0.990$) exists, in global terms, between total and cumulative fast-kinetics antiradical activities, and the adjusted values of the parameters were $m = 0.943$ y $r_t = 0.463$ (mmol L^{-1} molar equivalents of ascorbic acid). Thus, the total antiradical activity of a mandarin juice seems to be mostly due to its fast-kinetics antiradical activity. The relative contribution of A_1 to A_t ranged from about 75% for Clauselline and Fortune and 82% for Ortanique to 90% for the pure clementine (Clemenules, Marisol and Hernandine) and the hybrid Nova. The average value of this contribution for all these assayed cultivars was 87 ± 6.34%.

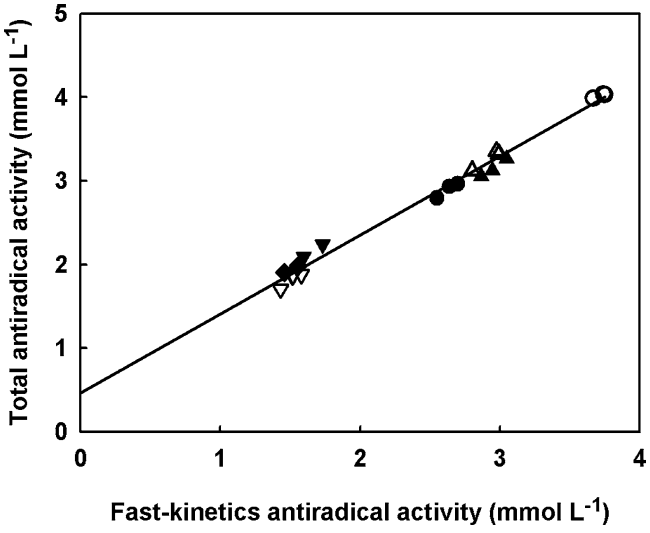

Fig. 2 Graphical representation of the total antiradical activity (A_t) vs. the cumulative fast-kinetics antiradical activity (A_1) for all the assayed juices: CMN (●), HRN (○), MRS (▲), NOV (△), FRT (▼), ORT (▽) and CLS (◆)

Ascorbic acid content and cumulative fast-kinetics antiradical activity

Figure 3 shows the graphical representation of the cumulative fast-kinetics antiradical activity, A_1 (mmol L^{-1} molar equivalents of ascorbic acid) vs. the concentration of ascorbic acid, c_o (mmol L^{-1}), for all the assayed juices. Experimental data points were fitted using the straight line $A_1 = c_o + r_1$, since in this case the coefficient of c_o must be 1 due to the definition of the unit of antiradical activity. The correlation

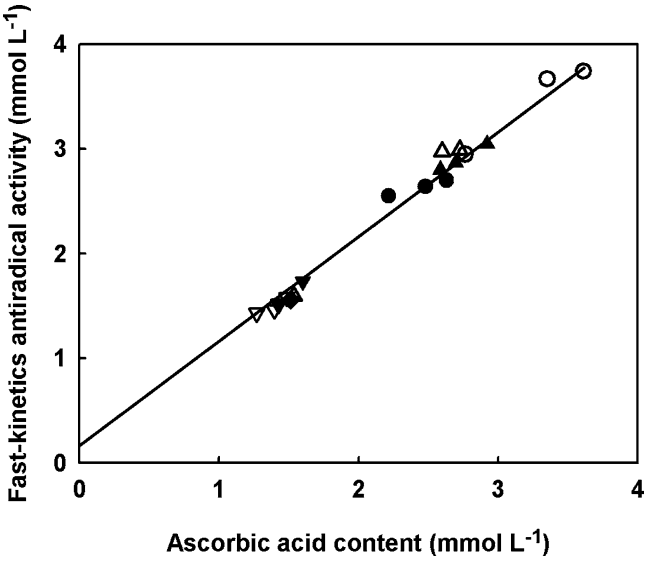

Fig. 3 Graphical representation of the cumulative fast-kinetics antiradical activity (A_1) vs. ascorbic acid concentration (c_o) for all the assayed juices: CMN (●), HRN (○), MRS (▲), NOV (△), FRT (▼), ORT (▽) and CLS (◆)

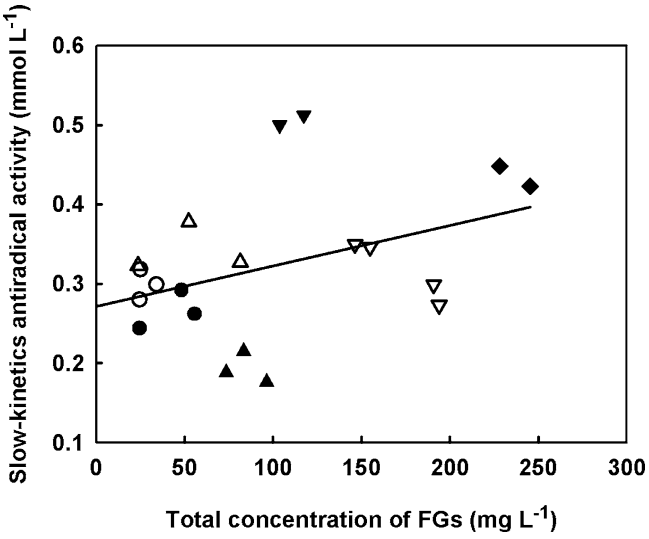

Fig. 4 Graphical representation of the cumulative slow-kinetics antiradical activity (A_2) vs. total FGs content for all the assayed juices: CMN (●), HRN (○), MRS (▲), NOV (△), FRT (▼), ORT (▽) and CLS (◆)

was quite good ($r^2 = 0.987$) and the value of the independent term, $r_1 = 0.161$ (mmol L^{-1} molar equivalents of ascorbic acid), was rather small. Therefore, it seems that ascorbic acid is by far the major contributor ($\approx 93.5 \pm 3.1\%$) to the fast-kinetics antiradical activity in mandarin juices. It is obvious, however, that there are some other minor contributors to this activity, most probably polyphenolic derivatives carrying a free *p*-catechol group (i.e. chlorogenic acid). On the other hand, the differences in the ascorbic acid content within the assayed juices were significant. As it can be seen from Table 2, or Fig. 3, the clementine Hernandine stood out by its high content in both ascorbic ($c_o > 3$ mmol L^{-1}) and dehydroascorbic (>0.1 mmol L^{-1}) acids, followed by Clemenules, Marisol and Nova ($2 < c_o < 3$ mmol L^{-1}), and Fortune, Ortanique and Clauselline ($1 < c_o < 2$ mmol L^{-1}).

Taking into account that ascorbic acid is the major contributor to the cumulative fast-kinetics antiradical activity, which is, in its turn, the major contributor to the total antiradical activity, it seems evident that ascorbic acid is also the major contributor to the total antiradical activity in all the assayed juices. This contribution ranges from about 70% for Clausellina juice to about 90% for clementine juices, with an average value of $81 \pm 3\%$. Moreover and as it was expected, these results are similar to those published for juices from non-pigmented (blood) oranges.

FGs content and cumulative slow-kinetics antiradical activity

Figure 4 shows the graphical representation of the cumulative slow-kinetics antiradical activity, A_2 (mmol L^{-1} molar equivalents of ascorbic acid) vs. the total concentration (mg L^{-1}) of FGs [25] for all the assayed juices. Experimental data were fitted using a straight line and the correlation found was rather weak ($r^2 = 0.151$). Therefore and taking into account the small contribution of the juice components belonging to the fast + slow-kinetics group to the cumulative fast-kinetics antiradical activity, it seems that mandarin juices contain other components (i.e. *p*-coumaric acid derivatives and some carotenoids) that significantly contribute to their cumulative slow-kinetics antiradical activity. Really, this result is not surprising at all since any component of the juice carrying a free phenol group is a potential contributor to this cumulative slow-kinetics antiradical activity.

It is noteworthy from data given in Table 2 that, as a general rule, the higher the cumulative fast-kinetics antiradical activity, A_1, the lower the cumulative slow-kinetics antiradical activity, A_2, and vice versa. Since the cumulative fast-kinetics antiradical activity mainly depends on the ascorbic acid content and the FGs are, without doubt, contributors to the slow-kinetics antiradical activity, data given in Table 2 also indicate that in mandarin juices the higher the concentration of ascorbic acid the lower the concentration of total FGs. Figure 5 shows the graphical representation of the total concentration (mg L^{-1}) of FGs vs. the concentration (mmol L^{-1}) of ascorbic acid for all the assayed juices. As it can be seen, the correlation was quite significant ($r^2 = 0.658$).

Statistical multivariate discriminant analysis

Cumulative fast-kinetics and slow-kinetics antiradical activities, A_1 and A_2, as well as its quotient, A_1/A_2, were assayed as variables for statistical multivariate discriminant

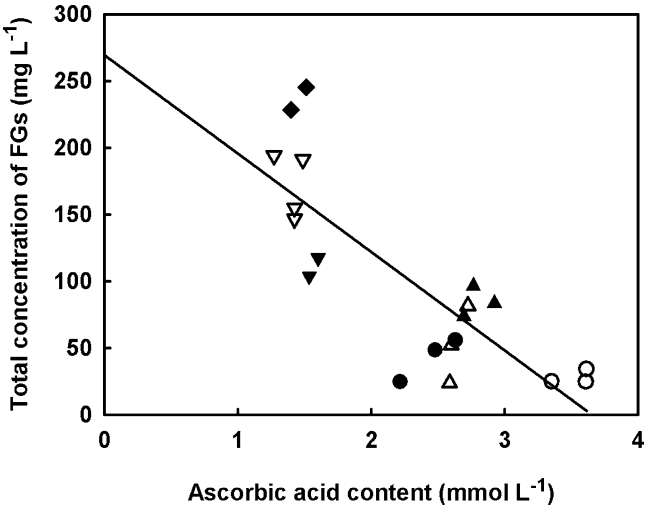

Fig. 5 Graphical representation of the total FGs content vs. ascorbic acid concentration (c_o) for all the assayed juices: CMN (●), HRN (○), MRS (▲), NOV (△), FRT (▼), ORT (▽) and CLS (♦)

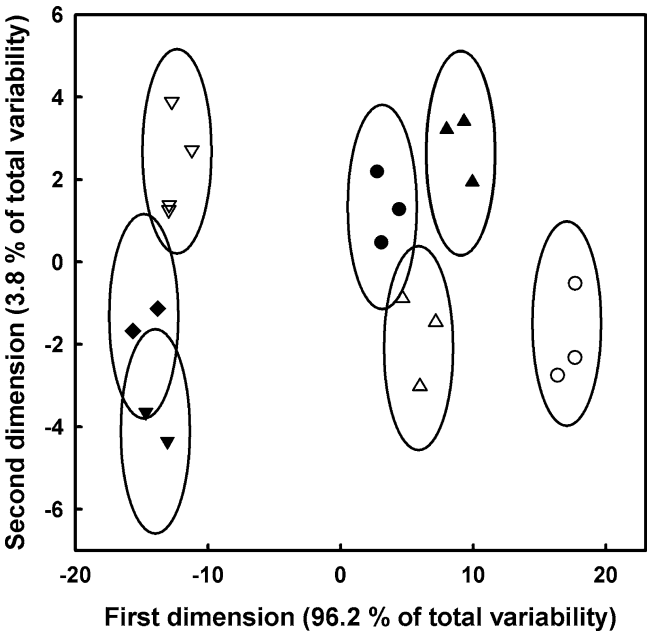

Fig. 6 Representation of varieties and confidence limits (95%) according to the canonical variables: CMN (●), HRN (○), MRS (▲), NOV (△), FRT (▼), ORT (▽) and CLS (♦)

analysis. Table 3 gives the main results from this analysis using standardized variables. As it can be seen, the quotient A_1/A_2 does not appear in Table 3, indicating that both cumulative fast-kinetics and slow-kinetics antiradical activities could completely discriminate all the assayed juices at a probability of 0.05 (significant distances of Mahalanobis between centroids). Figure 6 shows the graphical representation of samples according to the canonical variables, as well as the confidence limits (± 2.85). Data to calculate the canonical variables, X_1 and X_2, from the original variables A_1 and A_2 are as follows: mean value of A_1, $\bar{A}_1 = 2.449$; mean value of A_2,$\bar{A}_2 = 0.321$; and matrix of factor loadings for non-standardized variables $a_{11} = 13.36$, $a_{12} = -2.33$, $a_{21} = -18.32$ and $a_{22} = -31.49$. Thus, the canonical variables X_i ($i = 1,2$) are given by $X_i = (A_1 - \bar{A}_1)a_{1i} + (A_2 - \bar{A}_2)a_{2i}$. On the other hand, taking into account from data given in Table 3 that the second canonical variable almost depends exclusively on the cumulative slow-kinetics antiradical activity (A_2), this canonical representation could be replaced, with a minimum loss of efficiency, by two independent univariate analyses of the weak correlated original variables ($r^2 = 0.269$). Figure 7 shows the bivariate representation of samples according to the original variables, A_1 and A_2, as well as their confidence limits, 0.17 and 0.049, respectively.

Results of this work indicate that the total antiradical activity of juices from pure clementine (CMN, HRN and MRS), and consequently their content in ascorbic acid, was higher by far than that of the juice from the satsuma CLS. In contrast, their cumulative slow-kinetics antiradical activity and total FGs content were significantly smaller. Concerning the behaviour of the hybrids, NOV could be considered as an almost pure clementine, ORT resembles more an orange than a mandarin, and FRT behaves very similarly to the satsuma CLS.

Finally, results from the statistical multivariate discriminant analysis indicate that the cumulative fast-kinetics and slow-kinetics antiradical activities, A_1 and A_2, can be used as parameters to characterize and discriminate all the assayed juices. Nevertheless, a point must be clarified. These discriminant parameters have been obtained from fresh hand-squeezed juices. However, commercial juices are obtained using industrial extractors and they can subsequently be

Table 3 Results from the statistical multivariate discriminant analysis

Step	Entered original variable[a]	Wilk's lambda[b]	Approximate[b] F	Correct classifications	Factor loadings[c]	
					First canonical variable	Second canonical variable
1	A_1	$0.0063_{1,6,13}$	$343.2_{6,13}$	15	1.05	− 0.18
2	A_2	$0.0005_{2,6,13}$	$86.1_{12,24}$	20 (100%)	− 0.54	− 0.92

[a]In each sep, the variable entered and the previously entered variable were used

[b]Degrees of freedom shown as subindexes

[c]For standardized original variables

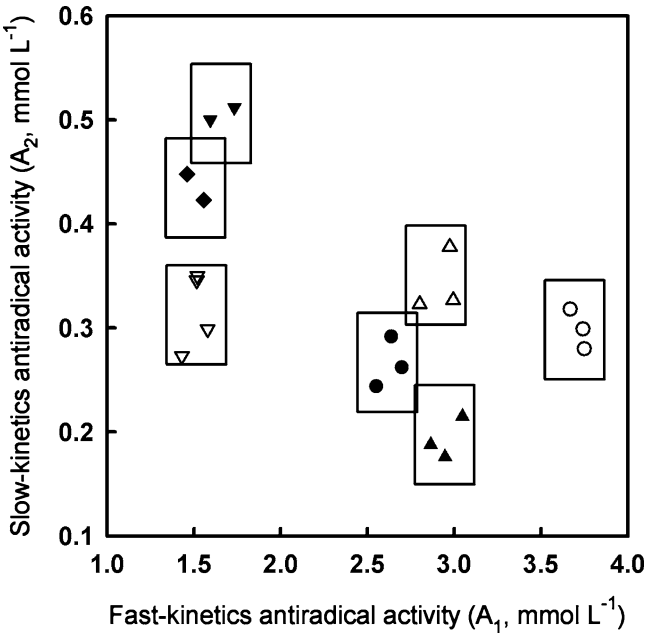

Fig. 7 Representation of varieties and confidence limits (95%) according to the original variables: CMN (●), HRN (○), MRS (▲), NOV (△), FRT (▼), ORT (▽) and CLS (◇)

subjected to different industrial processes (i.e., pasteurization, concentration, storage, etc.). Since the cumulative fast-kinetics antiradical activity mainly depends on the ascorbic acid content of the juice, the validity of this variable as a good characterizing parameter, after a given industrial process, will strongly depend on the effect of this process on the ascorbic acid concentration. Concerning to the cumulative slow-kinetics antiradical activity of the juices and since this activity seems to depend on much more stable components, it is to be expected that its discriminant power, although much more limited, will hold valid after most of the usual industrial processes.

Acknowledgements This research was supported by the Ministerio de Educación y Ciencia (Spain), project AGL2002-01172ALI, and AGROALIMED (Consellería de Agricultura, Pesca i Alimentació, Generalitat Valenciana, Spain).

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