Antioxidant Effectiveness As Influenced by Phenolic Content of Fresh Orange Juices

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Several fresh orange juices, obtained from five different *Citrus sinensis* (L.) Osbeck varieties (three pigmented varieties, Moro, Sanguinello, and Tarocco, and two blond varieties, Valencia late and Washington navel), were subjected to antioxidant profile determination (including total polyphenols, flavanones, anthocyanins, hydroxycinnamic acids, and ascorbic acid). The antioxidant activity of these juices was then assessed by means of different "in vitro" tests (bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical; peroxidation, induced by the water-soluble radical initiator 2,2′-azobis(2-amidinopropane) hydrochloride, on mixed dipalmitoylphosphatidylcholine/linoleic acid unilamellar vesicles; scavenging activity against nitric oxide; total antioxidant status). All orange juices tested showed an evident antioxidant effect. Our findings indicate the following: (1) the antioxidant efficiency of orange juices may be attributed, in a significant part at least, to their content of total phenols, (2) while ascorbic acid seems to play a minor role; (3) the antioxidant activity of orange juices is related not only to structural features of phytochemicals contained in them, but also to their capability to interact with biomembranes; (4) finally, as to pigmented juces, their antioxidant efficiency appears to be widely influenced by the anthocyanin level. One could speculate that the supply of natural antioxidant phenols through daily consumption of orange juice might provide additional protection against in vivo oxidation of cellular biomolecules.

Keywords: *Orange juice; phenols; antioxidant; anthocyanins*

INTRODUCTION

There is convincing epidemiologic evidence that the consumption of fruits and vegetables is in general beneficial to health and contributes to the prevention of degenerative processes, particularly lowering incidence and mortality rate of cancer and cardio- and cerebrovascular diseases (Hertog et al., 1993, 1995). The protection that fruits and vegetables provides against these diseases has been attributed to the various antioxidant phytonutrients contained in these foods. It is generally assumed that free radicals cause oxidative damage to lipids, proteins, and nucleic acids; therefore, antioxidants that can neutralize free radicals may be of central importance in the prevention of these disease states (Rice-Evans and Diplock, 1993).

In the past few years, an increasing interest in plant polyphenols, which are frequently components of the human diet, has been manifested. In fact, most of the antioxidant capacity of a fruit or vegetable may be from compounds other that vitamin C, vitamin E, or β-carotene, and plant polyphenols (such as flavonols, flavanols, anthocyanins, and phenylpropanoids) may act as antioxidants or as agents of other mechanisms contributing to anticarcinogenic or cardioprotective action (Castelluccio et al., 1995; Rice-Evans et al., 1996). The antioxidant potential of these compounds is dependent on the number and arrangement of the hydroxyl groups and the extent of structural conjugation, as well as the presence of electron-donating and electron-withdrawing substituents in the ring structure (Cao et al., 1997; Rice-Evans et al., 1996).

The juice from fruits of *Citrus sinensis* (L.) Osbeck is characterized by substantial accumulation (apart from ascorbic acid) of flavonoids and phenylpropanoids (Rapisarda et al., 1998; Rouseff et al., 1987). Particularly, the presence of anthocyanins is typical of blood orange varieties (Moro, Tarocco, Sanguinello varieties); in fact, the red color of blood orange peels and pulp is due to these pigments (Maccarone et al., 1985, 1999).

The health-related properties of polyphenols contained in orange juice (Benavente-García et al., 1997; Meyer et al., 1998) are based on their antioxidant activity. Recent studies have attempted to quantify the antioxidant capacity in foods, and a significant antioxidant activity was demonstrated to be exerted, in in vitro experimental systems, by commercial orange juices (Miller and Rice-Evans, 1997; Wang et al., 1996). However, no data are reported in the literature about the antioxidant potential of fresh orange juices; moreover, the orange phenolic profile shows quantitative differences as a function of the cultivars, environmental conditions of growing, and fruit maturity (Mouly et al.,

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Table 1. Codes and Harvest Dates of Orange Juices Tested

codes	orange varieties and clones[a]	harvest date
Moro I	Moro OL 8D	Dec 10
Moro II	Moro OL 8D	Jan 12
Moro III	Moro OL 8D	Feb 12
Moro IV	Moro OL 8D	Mar 10
Tarocco I	Tarocco NL 61−1E−A	Feb 12
Tarocco II	Tarocco OL 1E	Feb 12
Tarocco III	Tarocco OL "Tringali"	Feb 12
Tarocco IV	Tarocco OL "Gallo"	Feb 12
Tarocco V	Tarocco OL "Rosso"	Feb 12
Sanguinello I	Sanguinello OL5 "Moscato"	Feb 12
Sanguinello II	Sanguinello OL5 "Moscato"	Mar 9
Sanguinello III	Sanguinello OL5 "Moscato"	Apr 9
Valencia late	Valencia late NL "Olinda"	April 9
Washington navel	Washington navel NL 3330	March 9

[a] OL = old line; NL = new line.

1997; Rapisarda et al., 1998), so that the antioxidant capacity might vary accordingly.

Therefore, we have undertaken the study reported in the present paper to evaluate the antioxidant potential of fresh orange juices in relation to their quantitative/qualitative phenolic content. With this aim, several fresh orange juices, obtained from five different *Citrus sinensis* (L.) Osbeck varieties (three pigmented varieties, Moro, Sanguinello, and Tarocco, and two blond varieties, Valencia late and Washington navel), were selected and their antioxidant profile (including total phenols, flavanones, anthocyanins, hydroxycinnamic acids, and ascorbic acid) determined. The antioxidant activity of these juices was then assessed by means of different in vitro tests and the resulting values were correlated with each one of these classes of antioxidant compounds.

MATERIALS AND METHODS

Sample Preparation. Orange juices were obtained from fruits harvested, in the period December 1996−April 1997, at the Palazzelli experimental farm of the Istituto Sperimentale per l'Agrumicoltura (Acireale) in the territory of Lentini (Siracusa). The fruits of each clone were systematically sampled from three different plants. Codes and harvest dates are reported in Table 1. The oranges of each plant were halved and rapidly squashed by using a domestic squeezer, and then the juice obtained was filtered through 2 , 1, and 0.5 mm steel sieves successively and stored at −20 °C until used. No food preservative was added.

Total Phenols and Antioxidant Profile. The orange juices were analyzed for their total phenolic content according to the Folin-Ciocalteu colorimetric method (Di Stefano and Guidoni, 1989); total phenols were expressed as μg/mL ferulic acid equivalents. The antioxidant profile (flavanones, anthocyanins, hydroxycinnamic acids, ascorbic acid) was determined by HPLC as previously reported (Rapisarda and Intelisano, 1996; Rapisarda et al., 1994, 1998; Rouseff et al., 1997).

Quenching of DPPH (DPPH Test). The free-radical-scavenging capacity of orange juices was tested as bleaching of the stable 1,1-diphenyl-2-picrylhydrazyl radical (DPPH) (Saija et al., 1998). The reaction mixture contained, in 3.5 mL of ethanol, 86 μM DPPH and different amounts of orange juices. After 10 min at room temperature, the absorbance was recorded at 517 nm. All experiments were carried out in triplicate and repeated at least three times. Results were expressed as percentage decrease with respect to control values; mean scavenging concentrations (SC_{50}) and 95% confidence limits (95% C.L.) were calculated by using the Litchfield and Wilcoxon test (Tallarida and Murray, 1984).

Linoleate Peroxidation in LA/DPPC LUVs (LP-LUV Test). The method consists of the spectrophotometric determination of the accumulation of products (conjugated dienes; LOOH) of peroxidation, induced by the water-soluble radical initiator 2,2′-azobis(2-amidinopropane) hydrochloride (AAPH), on mixed dipalmitoylphosphatidylcholine/linoleic acid (DPPC/LA, molar ratio 1:0.125) unilamellar vesicles (LUVs) (Castelli et al., 1997; Saija et al., 1998). Different amounts of orange juices were added to 1.2 mL of LUV suspension (21 mg DPPC/mL). Then the mixture was incubated for 20 min at 37 °C in a shaking water bath. Then the radical initiator AAPH was added to the suspension to obtain a final concentration of 10 μM. The oxidation was carried out at 37 °C under air. At given time points (5−90 min), 120 μL aliquots of the reaction mixtures were withdrawn and added to 1 mL of methanol. The accumulation of LOOH formed from LA was evaluated by measuring the absorbance of the samples at 234 nm. The ratio of oxidation-induced change in absorbance with and without antioxidant addition was used to calculate a % inhibition of oxidation by the following equations:

$$[\mathrm{LOOH}]_{\mathrm{CONTR}} = (A_{90} - A_5)/(ET_{\mathrm{SEC}})$$

$$[\mathrm{LOOH}]_{\mathrm{ANTIOX}} = (A'_{90} - A'_5)/(ET_{\mathrm{SEC}})$$

$$\%\ \mathrm{inhibition} = ([\mathrm{LOOH}]_{\mathrm{CONTR}} - [\mathrm{LOOH}]_{\mathrm{ANTIOX}}/[\mathrm{LOOH}]_{\mathrm{CONTR}}) \times 100$$

where A_{90} and A'_{90} = absorbance at 90 min, respectively, without and with orange juice addition, A_5 and A'_5 = absorbance at 5 min, respectively, without and with orange juice addition, E (molar extinction coefficient of LOOH) = 26 100 ± 400 M^{-1} cm^{-1}, T_{SEC} (time, s) = 5100, and $[\mathrm{LOOH}]_{\mathrm{CONTR}}$ and $[\mathrm{LOOH}]_{\mathrm{ANTIOX}}$ = hydroperoxide concentration, respectively, without and with orange juice addition.

All experiments were carried out in triplicate and repeated at least three times. Results were expressed as percentage decrease with respect to control values; mean inhibitory concentrations (IC_{50}) and 95% confidence limits (95% C.L.) were calculated by using the Litchfield and Wilcoxon test (Tallarida and Murray, 1984).

Scavenging Activity against Nitric Oxide (NO Test). Nitric oxide interacts with oxygen to produce stable products, nitrite, and nitrate. Scavengers of nitric oxide compete with oxygen, leading to a reduced production of nitrite. The concentration of nitrite in aqueous solution can be assayed spectrophotometrically by using the Greiss reagent, with which nitrite reacts to give a stable product absorbing at 542 nm (Marcocci et al., 1994; Saija et al., 1999).

Briefly, sodium nitroprusside solution was prepared immediately before the experiment, dissolving 10 mM sodium nitroprusside in 20 mM phosphate buffer, pH 7.4, previously bubbled with argon. Orange juice was diluted in 20 mM phosphate buffer, pH 7.4, to obtain the opportune concentrations.

Greiss reagent solution was obtained by preparing a solution A, containing 2% (% w/v) sulfanilamide and 4% (% w/v) H_3PO_4, and a solution B, containing 0.2% (% w/v) naphthylethylenediamide. Immediately before the assay, 50 mL of solution A was added to 50 mL of solution B.

At the beginning of the experiment, 0.5 mL of diluted orange juice (at various concentrations) was diluted with 0.5 mL of sodium nitroprusside solution and incubated at 25 °C for 150 min. At the end of the incubation, 1 mL of Greiss reagent was added to each sample, and the absorbance was read at 542 nm. The nitrite concentration was calculated by referring to the absorbance of standard solutions of potassium nitrite. All experiments were carried out in triplicate and repeated at least three times. Results were expressed as percentage nitrite production with respect to control values (orange juice: 0 μL). The slope of the plot of percentage nitrite production vs orange juice volume was calculated by first-order exponential regression analysis, and the antioxidant efficiency (AE) was arbitrarily assumed as (−slope) × 100.

Total Antioxidant Status (TAA Test). The TAA is estimated using the ferrylmyoglobin/ABTS method for total antioxidant activity (Rice-Evans and Miller, 1994). This technique measures the relative ability of antioxidant sub-

Table 2. Antioxidant Profile of Tested Orange Juices[a]

orange juices	total phenols[b] (μg/mL)	total anthocyanins[c] (μg/mL)	total flavanones[d] (μg/mL)	total hydroxycinnamic acids[e] (μg/mL)	ascorbic acid (μg/mL)
Moro I	673.9 ± 18.5	97.5 ± 10.81	260.1 ± 23.3	63.3 ± 8.5	500.2 ± 25.2
Moro II	866.2 ± 17.3	167.2 ± 15.25	355.2 ± 29.9	60.1 ± 9.8	470.5 ± 21.7
Moro III	995.5 ± 28.2	222.3 ± 18.59	422.3 ± 44.8	140.2 ± 15.3	480.8 ± 23.0
Moro IV	1147.2 ± 34.9	278.4 ± 15.47	444.5 ± 51.7	135.4 ± 12.7	510.2 ± 26.5
Tarocco I	387.3 ± 12.7	1.2 ± 0.19	179.4 ± 15.4	38.0 ± 4.2	570.6 ± 29.3
Tarocco II	907.1 ± 29.4	70.4 ± 9.30	166.6 ± 17.8	70.1 ± 9.2	781.4 ± 33.4
Tarocco III	1090.5 ± 32.6	99.4 ± 10.46	150.2 ± 14.5	91.2 ± 10.0	752.0 ± 34.6
Tarocco IV	569.3 ± 23.5	4.2 ± 0.58	180.4 ± 19.0	86.6 ± 10.5	570.3 ± 31.6
Tarocco V	470.1 ± 22.9	20.9 ± 1.68	171.6 ± 19.3	66.3 ± 8.4	690.7 ± 30.9
Sanguinello I	382.5 ± 15.2	6.4 ± 1.16	185.7 ± 20.6	46.4 ± 5.8	515.5 ± 26.1
Sanguinello II	560.6 ± 20.3	41.4 ± 3.94	285.1 ± 30.5	72.7 ± 9.7	535.2 ± 25.8
Sanguinello III	602.9 ± 21.6	52.6 ± 6.77	300.2 ± 28.2	92.1 ± 10.6	490.0 ± 24.6
Valencia late	488.3 ± 19.7		244.1 ± 19.6	56.9 ± 4.8	576.8 ± 30.5
Washington navel	361.4 ± 16.9		202.3 ± 21.3	33.4 ± 4.9	417.0 ± 18.3

[a] Each result is expressed as mean ± S.D. of three samples. [b] As ferulic acid equivalents. [c] As cyanidin-3-glucoside. [d] Hesperidin and narirutin. [e] Caffeic, ferulic, synapic, and *p*-coumaric acids.

Table 3. Antioxidant Activity of Orange Juices Tested in Different Experimental Models

	antioxidant effectiveness			
orange juices	DPPH test SC_{50} (95% C.L.) (μL)	TAA[a] Trolox equiv (mM)	LP-LUV test IC_{50} (95% C.L.) (μL)	NO test (AE)
Moro I	46.35 (38.07−56.41)	4.94 ± 0.05	10.92 (9.44−12.61)	0.0156
Moro II	37.61 (31.83−44.49)	5.81 ± 0.08	4.89 (3.87−6.20)	0.0313
Moro III	27.56 (22.13−32.75)	6.11 ± 0.09	4.44 (3.37−5.29)	0.0335
Moro IV	25.44 (20.79−29.57)	7.05 ± 0.08	4.11 (3.25−5.18)	0.0388
Tarocco I	48.66 (38.98−60.74)	3.84 ± 0.10	11.78 (9.12−15.23)	0.0102
Tarocco II	24.28 (21.34−27.64)	6.62 ± 0.09	11.79 (10.20−13.63)	0.0330
Tarocco III	27.12 (21.93−32.48)	5.30 ± 0.07	11.22 (9.94−12.78)	0.0210
Tarocco IV	49.31 (40.13−58.76)	4.06 ± 0.07	12.13 (10.63−13−44)	0.0153
Tarocco V	47.94 (39.06−56.27)	3.76 ± 0.05	13.35 (10.32−16.80)	0.0160
Sanguinello I	80.01 (62.40−103.50)	1.03 ± 0.09	13.26 (10.41−16.88)	0.0178
Sanguinello II	49.74 (40.26−59.18)	4.39 ± 0.09	13.07 (11.48−14.53)	0.0203
Sanguinello III	40.17 (32.84−48.55)	4.50 ± 0.10	12.88 (11.15−14.27)	0.0225
Valencia late	50.48 (40.75−62.49)	0.74 ± 0.04	22.38 (19.92−24.99)	0.0051
Washington navel	67.68 (52.74−86.86)	1.13 ± 0.05	20.58 (19.18−22.07)	0.0058

[a] Mean ± S.D. of three samples.

stances to scavenge the 2,2′-azinobis(3-ethylbenzothiazoline-6-sulfonate) radical cation ($ABTS^{\bullet+}$), compared with standard amounts of the synthetic antioxidant Trolox, the water soluble vitamin E analogue. The radical cation $ABTS^{\bullet+}$, generated in the aqueous phase from ABTS through the peroxidation action of metmyoglobin, is a blue/green chromogen with characteristic absorption at 734 nm. The evaluation of the TAA was carried out using the RANDOX kit (Randox Laboratories Ltd., Ardmore, U.K.). Each determination was performed in triplicate and repeated at least three times. Results are expressed as mean ± S.D. of three samples.

RESULTS AND DISCUSSION

The content of total phenols, anthocyanins, flavanones, hydroxycinnamic acids, and ascorbic acid in orange juices is given in Table 2. The levels of total phenols as determined by the Folin−Ciocalteu method varied from 361.4 ± 16.9 to 1147.2 ± 34.9 μg/mL ferulic acid equivalents. The content of flavanones (the most abundant being hesperidin and narirutin), hydroxycinnamic acids (represented by ferulic, caffeic, synapic, and *p*-coumaric acids), and ascorbic acid ranged, respectively, from 150.2 ± 14.5 to 444.5 ± 51.7 μg/mL, from 33.4 ± 4.9 to 140.2 ± 15.3 μg/mL, and from 417 ± 18.3 to 781 ± 33.4 μg/mL. As expected, only the blood orange juices contained appreciable values of anthocyanins (principally cyanidin-3-glucoside), ranging from 1.2 ± 0.19 to 278.4 ± 15.47 μg/mL.

The results obtained in the four tests employed in our research are reported in Table 3. However, a brief methodological comment is needed before discussing these data. In the present study, the antioxidant effectiveness of orange juices was evaluated by means of different tests, in homogeneous organic (DPPH test) or aqueous (TAA) phase or in a membranous system (LH/DPPC LUV test), besides that in the NO test (which can give information about a specific target of the antioxidants contained in the orange juice). First, it is known that, given the reactivity of an antioxidant in homogeneous solution, the actual antioxidant effect in membranes can either increase or decrease due to several factors (drug partitioning between the aqueous phase and the lipid phase, drug-induced membrane stabilizing or destabilizing effects, etc.) that are not considered in chemical tests. Second, the apparent antioxidant efficiency of a drug may be influenced by its solubility in the reaction medium; biophenols contained in the orange juice are present in a partially dissolved/partially suspended/partially colloidal form, so that only the activity of the dissolved fraction can be measured by employing an aqueous system. Finally, the antioxidant activity of a drug may be greatly affected by the system used as substrate and the conditions used to catalyze oxidation (Satué-Gracia et al., 1997).

All orange juices tested in our study showed an evident antioxidant effect (see Table 3). Furthermore, concerning those obtained from pigmented varieties, the orange juices with higher anthocyanin levels were, in

Table 4. Correlation Coefficients of Linear Regression between the Concentration of Individual Antioxidant Constituents to the Antioxidant Activity of Tested Orange Juices

antioxidant components (μg/mL)	correlation coefficient (R) DPPH test SC_{50} (μL)	TAA mM Trolox	LP-LUV test IC_{50} (μL)	NO test (AE)
total phenols	0.866	0.831	0.724	0.821
total anthocyanins	0.666	0.760	0.827	0.845
total flavanones	0.425	0.491	0.616	0.632
total hydroxycinnamic acids	0.698	0.674	0.656	0.727
ascorbic acid	0.330	0.245	0.065	0.091

each test employed, better antioxidants than those with lower anthocyanin content.

A direct correlation between the antioxidant effectiveness of orange juices and their total phenol content was demonstrated by linear regression analysis. In fact, as reported in Table 4, high correlation levels were calculated in all tests employed ($R = 0.866$ in the DPPH test; $R = 0.831$ in the TAA test; $R = 0.724$ in the LP-LUV test; $R = 0.821$ in the NO test). Good correlation levels were observed also for hydroxycinnamic acids, flavanones, and anthocyanins, with the ascorbate fraction playing a minor role. Figure 1 shows the best correlation calculated, for each test employed in our study, when the antioxidant efficiency has been correlated with each one of the classes of antioxidant compounds taken into consideration; for reasons of clarity, only the mean antioxidant concentrations of each clone are reported in the graphics of Figure 1.

The findings reported herein allow us to emphasize some issues: (1) the antioxidant efficiency of orange juices may be attributed, in a significant part at least, to their content of total phenols, (2) while ascorbic acid seems to play a minor role; (3) the antioxidant activity of orange juices is related not only to structural features of phytochemicals contained in them, but also to their capability to interact with biomembranes; (4) as to pigmented juces, their antioxidant efficiency appears to be widely influenced by the anthocyanin level.

As to the first issue, our study clearly demonstrates that the antioxidant activity of orange juices is not a property of a single phytochemical compound, but is widely distributed among the phenolic constituents. Similarly, phenolic phytochemicals are responsible for the antioxidant activity of wines (Frankel et al., 1995; Meyer et al., 1997; Simonetti et al., 1997; Vinson and Hontz, 1995) and have been hypothesized to act synergistically as antioxidants in a mechanism in which the easily oxidized phenols are regenerated by less active phenols (Kanner et al., 1994). More particularly, in

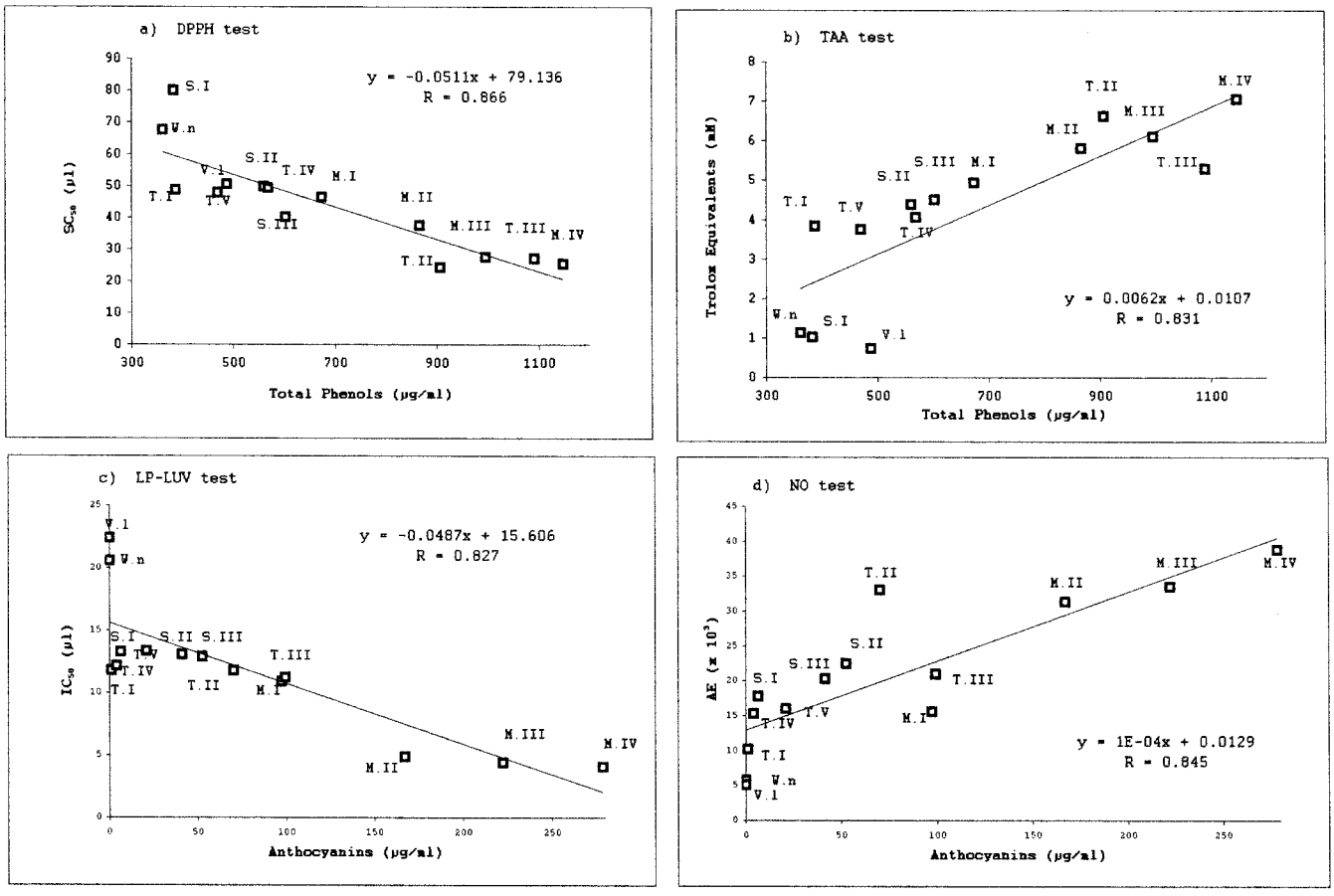

Figure 1. Relationship, calculated by linear regression analysis, for the tested orange juices from different varieties, between: (a) concentration of total phenols (μg/mL; *X)* and SC_{50} values (μL; *Y)* calculated in the DPPH test; (b) concentration of total phenols (μg/mL; *X)* and Trolox equivalents (mM; *Y)* calculated in the TAA test; (c) concentration of anthocyanins (μg/mL; *X)* and IC_{50} values (μL; *Y)* calculated in the LP-LUV test; (d) concentration of anthocyanins (μg/mL; *X)* and AE values *(Y)* calculated in the NO test. Values are the mean of three samples; relative standard deviations were in the range of 1.99−18.12% (see Table 2). Codes: M.I = Moro I; M.II = Moro II; M.III = Moro III; M.IV = Moro IV; T.I = Tarocco I; T.II = Tarocco II; T.III = Tarocco III; T.IV = Tarocco IV; T.V = Tarocco V; S.I = Sanguinello I; S.II = Sanguinello II; S.III = Sanguinello III; V.l = Valencia late; W.n = Washington navel. □ experimental; — calculated.

orange juice phenolic antioxidants proved to protect vitamin C against oxidative decomposition (Miller, 1998).

Second, the low correlation level calculated in each test for ascorbic acid is not surprising. For example, Wang and co-workers (1996) have recently evaluated that the contribution of vitamin C to the total antioxidant activity of a fruit is usually less than 15%. Moreover, Miller and Rice-Evans (1997) have underlined the significant contributory role of polyphenols (particularly hesperidin and narirutin) in the total antioxidant activity of long-life orange juice, even if vitamin C (which is added, however, during manufacture of this fruit beverage) was the most abundant antioxidant.

As to the third point, as evidenced by results obtained in the DPPH test and in the LP-LUV test, the better antioxidant efficiency calculated in the membranous system (LP-LUV test) than in homogeneous solution (DPPH test), as well as the different order of correlation coefficients, leads us to suppose that the antioxidant activity of the orange juice is very likely dictated not only by structural features of its phytochemical constituents but also by their capability to interact with biomembranes. In agreement with our hypothesis, flavonoids have been demonstrated to be located at the cellular membrane-lipid/water interface, where aqueous radicals are easily trapped, and are accessible to chain-initiating peroxyl radicals (Saija et al., 1995; Terao and Piskula, 1998).

Finally, it is evident that, apart that of total phenols, anthocyanin content (which varies depending on cultivars and fruit maturity stage) is the main factor influencing the antioxidant effectiveness of fresh pigmented juices. In accordance with our data, total phenolic and anthocyanin content appeared to be the main components in the antioxidant activity (as evaluated in different in vitro systems) of different berries (Prior et al., 1998) and red wines (Ghiselli et al., 1998). This is a very interesting result, because anthocyanins have been shown to have some positive therapeutic and protective effects, for example, in the treatment of diabetic retinopathy and of various microcirculation diseases, or as antineoplastic, antiinflammatory, and hepatoprotective agents (Wang et al., 1997). Besides being a potent scavenger of free radicals and reactive oxygen species (Rice-Evans et al., 1996), cyanidin is an excellent scavenger also of nitric oxide radical (van Acker et al., 1995). In particular, cyanidin-3-glucoside (the most abundant anthocyanin present in red orange juice) (Maccarone et al., 1985) was shown to have marked antioxidant activity in a linoleic acid autoxidation system (Tsuda et al., 1996) and to possess, in comparison with other anthocyanins, the highest oxygen radical absorbance capacity, 3.5 times stronger than that of Trolox (Wang et al., 1997).

On the basis of these findings, one could speculate that the supply of natural antioxidant phenols through daily consumption of orange juice might provide additional protection against in vivo oxidation of cellular biomolecules. As also supported by results from several authors (Abbey et al., 1995; So et al., 1996; Tsuda et al., 1998), the supplementation of natural antioxidants contained in the orange juice through a balanced diet could be more effective and also more economical than the supplementation of an individual antioxidant in protecting the body against oxidative damage under different conditions. However, further experiments are needed to clarify if orange juice can act as an antioxidant in vivo.

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