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Evaluation of antioxidant capacity of blood orange juices as influenced by constituents, concentration process and storage

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Abstract

Total Antioxidant Activities (TAA) of freshly squeezed and processed blood and blond orange juices were measured using the ABTS radical-cation method. Blood juices have TAA values higher than blond juices, and freshly-squeezed juices are higher than processed. Levels of ascorbic acid, hydroxycinnamic acid and anthocyanins were determined in order to calculate relative contributions to TAA by Trolox Equivalent Antioxidant Capacity (TEAC) of such constituents. Ascorbic acid was the main contributor (~70%), followed by hydroxycinnamic acids and anthocyanins. Calculated TAA accounted for 91% (mean) of that measured, reaching a maximum of 98% (mean) for the 'not-from-concentrate' blood juices (NFC). Reconstituted (from concentrate) blood juices (RFC) had higher TAA than NFC, and the difference could be ascribed to the increased amount of carotenoid pigments in the serum of RFC juices, as a consequence of the thermal concentration process. Despite the degradation of anthocyanins during storage, TAA of NFC and RFC juices remained unchanged up to 60 days at 2°C, whereas it decreased when RFC juice was stored at 20°C, in accordance with the observed decrease of ascorbic acid.

1. Introduction

Recently, there has been an increasing interest in antioxidants, both in vitro and in vivo (Scott, 1997). Several methods have been developed to evaluate the total antioxidant activity (TAA) of biological samples (Cao, Alessio, & Culter, 1993; Cao, Verdon, Wu, Wang, & Prior, 1995; Rice-Evans & Miller, 1994) and studies of the antioxidant capacity of fruits and vegetables have received especial attention since Ames, Shigena, and Hagen (1993) offered epidemiological evidence that fruit and vegetable consumption was positively correlated to health. In addition to the well-known vitamin C, vitamin E and carotenoids, other microconstituents contribute to the antioxidant capacity of fruits and vegetables (Cao et al., 1995; Miller, Diplock, & Rice-Evans, 1995; Wang, Cao, & Prior, 1996). The phenolic extracts from plant materials have antioxidant activity (Kahkonen et al., 1999) and many researchers have demonstrated the structure-antioxidant activity relationships of flavonoids, hydroxycinnamic acids, and coumarins (Chen & Ho, 1997; Foti, Piattelli, Baratta, & Ruberto, 1996; Natella, Nardini, Di Felice, & Scaccini, 1999). Anthocyanins also have a high antioxidant capacity, and they give a strong contribution to the antioxidant activity of small fruits (Kalt, Forney, Martin, & Prior, 1999; Prior et al., 1998) as well as grapes and red wines (Simonetti, Pietta, & Testolin, 1997; Tamura & Yamagami, 1994).

Wang et al. (1996), measuring the TAA of 12 fruits and five commercial fruit juices, including orange juice, found that the contribution of vitamin C to TAA, in many of them, was less than 15%, and in any case always less than 30%. Miller et al. (1995) and Miller and Rice-Evans (1997), studying the contribution to TAA in fruit juices, found that most important was ascorbic acid, except for apple juice, which was very poor in vitamin C. In orange juice, the contribution of ascorbic acid to TAA was about 87%. Recently, Gardner, White, McPhail, and Duthie (2000), monitoring the antioxidant activity with two different methods, assessed that the antioxidant activity in orange juices was mainly due to vitamin C (> 60%), according with the Miller and Rice-Evans (1997) results.

Blood orange juice is a typical Italian product. It contains anthocyanins (Maccarone, Maccarone, Perrini, &

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Rapisarda, 1983; Maccarone, Maccarrone, & Rapisarda, 1985; Maccarone, Rapisarda, Fanella, Arena, & Mondello, 1998; Rapisarda & Giuffrida, 1992) ascorbic acid (Rapisarda & Intelisano, 1996) and hydroxycinnamic acids (Rapisarda et al., 1998) at higher levels than blond orange juices. TAA of some Italian freshly-squeezed blood orange juice, has been assessed using four different methodologies by Rapisarda, Tomaino, Lo Cascio, Bonina, De Pasquale, and Saija (1999). They found that the major contribution was due the total content of phenols; TAA values appeared largely related to anthocyanin levels, while ascorbic acid seemed to play a minor role.

The present study was aimed to evaluate the effect of the thermal concentration process of blood orange juices on antioxidant capacity, to investigate the contribution of each antioxidant component to the TAA using the TEAC values and, lastly, to measure the change in antioxidant capacity of processed blood orange juice during storage.

2. Materials and methods

2.1. General analyses

In the present study, 17 orange juices have been analysed. Eight blood orange juices were drawn directly from the plants of Ruby International Company (Catania, Italy; samples 1–8): four of them before the concentration process (NFC juices) and four juices immediately after the concentration in a TASTE evaporator; the concentrated juices were reconstituted (RFC juices) with distilled water up to the same °Brix of the corresponding NFC juices (11.5-12 °Brix); five commercial juices were purchased in a supermarket (one blood and four blond juices, samples 9–13) and four orange juices were obtained from fresh fruits of a single cultivar (three blood and one blond, samples 14–17). The list of samples is shown in Table 1.

Two samples, a NFC and a RFC juice, were treated with sodium benzoate (200 mg/l), and a series of aliquots were bottled under sterile conditions in a flow laminar cabinet and stored at 2 and 20°C up to 60 days. Every 15 days, some aliquots were withdrawn and analysed for determining the TAA value, ascorbic acid and anthocyanin concentrations.

All juices were centrifuged at 7000 rpm for 20 minutes and the clear juice was used for analyses. Ascorbic acid was determined by reverse phase HPLC (Rapisarda & Intelisano, 1996), as also were the four hydroxycynnamic acids (Rapisarda et al., 1998); anthocyanin level was measured by spectrophotometry and expressed as milligrams per litre of cyanidin-3-glucoside, the predominant anthocyanin of blood orange juice (Rapisarda, Fallico, Izzo, & Maccarone, 1994; Rapisarda, Fanella, & Maccarone, 2000). HPLC solvents were Merck (Milan, Italy), all other chemicals were Sigma-Aldrich (Milan, Italy).

Table 1
Contents of ascorbic acid, anthocyanins and hydroxycinnamic acids (mg/l) in orange juices (mmol/l in parentheses)

Sample	Colour	Ascorbic acid	Anthocyanins	Sinapic acid	Caffeic acid	Ferulic acid	Coumaric acid
Processed juices							
1 NFC[a]	Blood	441 (2.50)	72.0 (0.15)	15.1 (0.07)	7.1 (0.04)	38.3 (0.20)	23.5 (0.14)
2 RFC[b]	Blood	482 (2.74)	68.9 (0.14)	14.8 (0.07)	6.5 (0.04)	39.1 (0.20)	22.7 (0.14)
3 NFC[a]	Blood	440 (2.50)	97.0 (0.20)	14.1 (0.06)	7.5 (0.04)	44.2 (0.23)	25.3 (0.15)
4 RFC[b]	Blood	445 (2.53)	104 (0.21)	14.7 (0.07)	7.3 (0.04)	43.8 (0.23)	22.0 (0.13)
5 NFC[a]	Blood	602 (3.42)	48.3 (0.10)	10.3 (0.05)	5.1 (0.03)	38.2 (0.20)	12.4 (0.08)
6 RFC[b]	Blood	535 (3.04)	49.0 (0.10)	10.5 (0.05)	4.9 (0.03)	40.1 (0.21)	14.1 (0.09)
7 NFC[a]	Blood	489 (2.78)	48.7 (0.10)	12.6 (0.06)	4.4 (0.02)	40.9 (0.21)	22.2 (0.14)
8 RFC[b]	Blood	535 (3.04)	57.2 (0.12)	12.0 (0.05)	5.0 (0.03)	41.1 (0.21)	21.5 (0.13)
Commercial juices							
9 NFC	Blood	518 (2.94)	65.7 (0.14)	10.3 (0.05)	5.1 (0.03)	36.8 (0.19)	22.6 (0.14)
10 NFC	Blond	319 (1.81)	–	8.5 (0.04)	3.5 (0.02)	38.3 (0.20)	7.4 (0.05)
11 NFC	Blond	262 (1.49)	–	6.7 (0.03)	3.2 (0.02)	30.1 (0.16)	16.5 (0.10)
12 RFC	Blond	321 (1.82)	–	8.5 (0.04)	3.9 (0.02)	35.1 (0.18)	15.5 (0.09)
13 RFC	Blond	350 (1.99)	–	7.5 (0.03)	4.5 (0.02)	37.4 (0.19)	16.30 (0.10)
Freshly-squeezed juices							
14 Moro	Blood	550 (3.12)	72.3 (0.15)	10.1 (0.05)	4.6 (0.03)	36.6 (0.19)	22.6 (0.14)
15 Tarocco	Blood	669 (3.80)	26.2 (0.05)	11.7 (0.05)	4.8 (0.03)	42.5 (0.22)	20.5 (0.13)
16 Tarocco	Blood	533 (3.02)	103 (0.21)	10.4 (0.05)	5.0 (0.03)	31.6 (0.16)	26.5 (0.16)
17 Vaniglia	Pink[c]	355 (2.01)	–	9.9 (0.04)	4.6 (0.03)	37.8 (0.20)	16.6 (0.10)

[a] Not from concentrated.
[b] Reconstituted to the same Brix of the corresponding NFC.
[c] Colour is due to carotenoids.

Table 2
Measured TAA values of orange juices, and calculated TAA from the contributions of ascorbic acid, anthocyanins and hydroxycinnamic acids

Sample[a] No.	TAA measd	TAA [b] calcd	Ascorbic ac.	Anthocyanins	Sinapic ac.	Caffeic ac.	Ferulic ac.	Coumaric ac.
Processed juices								
1	3.55	3.60	2.48	0.37	0.09	0.05	0.37	0.24
2	3.56	3.81	2.71	0.35	0.09	0.05	0.37	0.24
3	3.76	3.78	2.48	0.49	0.07	0.05	0.43	0.26
4	4.62	3.81	2.50	0.52	0.09	0.05	0.43	0.22
5	4.61	4.25	3.39	0.25	0.06	0.04	0.37	0.14
6	4.66	3.91	3.01	0.25	0.06	0.04	0.39	0.16
7	3.67	3.72	2.75	0.25	0.07	0.03	0.39	0.23
8	4.56	4.04	3.01	0.30	0.07	0.04	0.39	0.23
Commercial juices								
9	4.18	3.95	2.91	0.35	0.06	0.04	0.35	0.24
10	2.73	2.33	1.79	–	0.05	0.03	0.37	0.09
11	2.37	1.75	1.48	–	0.04	0.03	0.30	0.17
12	2.97	2.37	1.80	–	0.05	0.03	0.33	0.16
13	2.19	2.56	1.97	–	0.04	0.03	0.35	0.17
Freshly-squeezed juices								
14	5.08	4.16	3.10	0.37	0.06	0.04	0.35	0.24
15	5.18	4.61	3.76	0.12	0.06	0.04	0.41	0.22
16	5.09	4.19	2.99	0.52	0.06	0.04	0.30	0.28
17	2.38	2.62	1.99	–	0.05	0.04	0.36	0.18

[a] Key for number is that of Table 1.
[b] 0.99 mmol/l Ascorbic acid + 2.47 mmol/l Anthocyanins + 1.22 mmol/l Sinapic acid + 1.34 mmol/l Caffeic acid + 1.86 mmol/l Ferulic acid + 1.73 mmol/l Coumaric acid.

Standard 1 mmol/l solutions of ascorbic acid, caffeic acid, sinapic acid, ferulic acid and coumaric acid were prepared in order to determine their TEAC values according to Miller et al. (1995).

2.2. Assessment of antioxidant activity

TAA values, both in orange juices and in standard solutions, were measured by the ABTS [2,2′-Azino-di-(3-ethylbenzthiazoline-6-sulphonate)] radical cation decolourization method (Miller et al., 1995), measured at 600 nm using the RANDOX kit (Randox Ltd., Crumlin, UK). ABTS was incubated with a peroxidase enzyme (metmyoglobin) and H_2O_2 to produce the corresponding coloured radical cation of ABTS. This has a relatively stable blue-green colour. When an antioxidant was added to the sample there was a suppression in the formation of this colour, proportionally to its concentration. All results were expressed as mmol/l Trolox equivalent.

3. Results and discussion

Table 1 lists concentrations (both in mg/l and in mmol/l) of ascorbic acid, and the four hydroxycinnamic acids (sinapic, caffeic, ferulic and coumaric) in blood and blond orange juices and anthocyanins in blood juices. All the blood juices have higher amounts of ascorbic acid and hydroxycinnamic acids than blond ones. The thermal concentration process of blood orange juices did not lead to a decrease of ascorbic acid, anthocyanins or hydroxycynnamic acids, confirming findings of previous studies (Fallico, Lanza, Maccarone, Nicolosi Asmundo, & Rapisarda, 1996; Maccarone et al., 1996).

The Trolox Equivalent Antioxidant Capacity (TEAC) measurements of ascorbic acid and hydroxycinnamic acids gave the following values: 0.99 (ascorbic acid), 1.22 (sinapic acid), 1.34 (caffeic acid), 1.86 (ferulic acid), 1.73 (coumaric acid). The TEAC values of ascorbic, caffeic and ferulic acids are very similar to those obtained by Miller et al. (1995). For the cyanidin-3-glucoside, a TEAC value of 2.47 was used, as in the Miller and Rice Evans (1997) study.

Table 2 lists the measured values of TAA for all juices, together with the calculated TAA obtained from the sum of the contributions (TEAC value$\times C_{(mmol/l)}$) of ascorbic acid, anthocyanins and the four hydroxycinnamic acids. The mean value of TAA of blood orange juices (samples 1–9, 14–16) was higher than the TAA values of blond ones (samples 10–13, 17); mean values of 3.90 and 4.35 were obtained for NFC and RFC blood juices, and 2.57 for blond ones, respectively. The highest TAA values in blood juices cannot be ascribed only to the presence of anthocyanins (Table 2). Although anthocyanins have very high TEAC values,

their concentration in the analysed blood orange juices does not exceed 104 mg/l (0.21 mmol/l). They give a mean contribution to TAA of 0.35 (Table 2), comparable with that of ferulic or coumaric acids. In any case it seems to be always lower than the contribution of the four hydroxycynnamic acids (0.73). Both in blood and blond juices the most important contribution to the TAA is given by ascorbic acid, which represents about the 70% of TAA in both juices, reaching a TAA value of 2.78 in blood juices and 1.76 in blond. Higher TAA values in blood juices than blond can be ascribed to the contributions of different factors: the presence of anthocyanins, higher hydroxycinnamic acid levels, and, overall, the higher level of ascorbic acid (Table 1).

In blood RFC juices, compared with the corresponding NFC, a lower value of TAA was anticipated as a consequence of the thermal concentration process. Instead, a mean value of 4.35 was found, higher than the TAA value of the NFC juices. The calculated value of TAA strictly agrees with the measured TAA in NFC juices (Table 2), while the calculated TAA for RFC juices fails to explain about 10% of the measured TAA.

Recently, other studies carried out on tomato, coffee and tea have shown that a prolonged heating time enhanced the antioxidant capacity of these foods inducing the formation of compounds with antioxidant activity, e.g. Maillard's reaction products (MRPs; Nicoli, Anese, Parpinel, Franceschi, & Lerici, 1997; Nicoli, Anese, & Manzocco, 1999). On the other hand, previous studies carried out in blood orange juices have shown that the concentration process does not induce the formation of MRPs (Arena, Fallico, & Maccarone, 2001), but induces a different distribution of carotenoids between pulp and serum, and a modification of pulp structure (Arena, Fallico, & Maccarone, 2000). Furthermore, it is known that carotenoids have chain breaking activity and, overall, a quenching activity (Scott, 1997), so they can make their own contribution to the TAA of RFC juices, because of their high concentration. These results agree with the Wang et al. (1996) findings that, in most fruits, the contribution of the pulp fraction to antioxidant activity (expressed as ORAC activity), is usually less than 10%.

The three freshly-squeezed blood orange juices (samples 14–16) showed the highest TAA values (mean value 5.12). Also, in these cases, the juices had the highest ascorbic acid level (mean value 518 mg/l).

Fig. 1 shows the TAA results of two blood orange juices (NFC and the corresponding RFC) stored up to 60 days at 2 or 20°C. The TAA value is almost constant up to 60 days storage, with the exception of RFC juice stored at 20°C (Fig. 1A). In the same time interval, ascorbic acid showed a trend similar to the TAA values (Fig. 1B), whereas anthocyanin levels decreased very rapidly for samples stored at 20°C (Fig. 1C). These

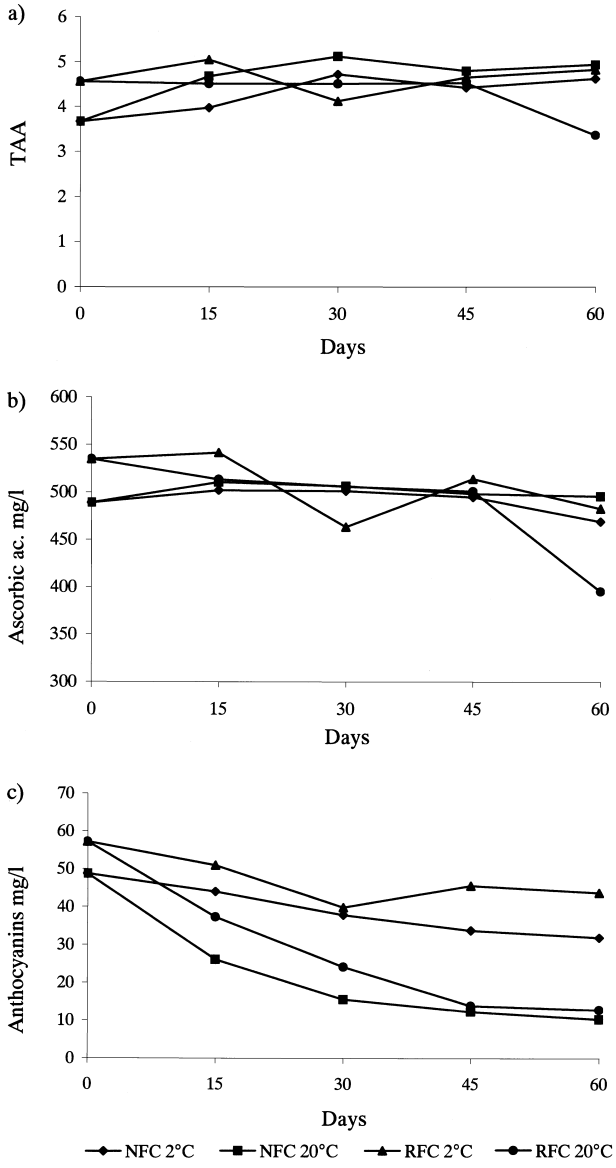

Fig. 1. (a) TAA values, (b) ascorbic acid and (c) anthocyanins during storage of NFC and RFC juices at 2 and 20°C.

results confirm that antioxidant capacity in orange juice is strictly related to ascorbic acid concentration.

Summarising, the antioxidant capacity, of blond and blood orange juices is due to the contribution of many factors. Ascorbic acid is the major contributor according to Miller et al. (1995), Miller and Rice Evans (1997) and Gardner et al. (2000). According to Rapisarda et al. (1999), the phenolic components, including hydroxycinnamic acids, make an important contribution to TAA values, but, the differences between blood and blond juices, are prevalently due to different levels of ascorbic acid. Anthocyanins in blood orange juices make a contribution to total antioxidant activity that does not exceed 10%.

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