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The relative contributions of vitamin C, carotenoids and phenolics to the antioxidant potential of fruit juices

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Abstract

The health benefit of fruit juices have been ascribed, in part, to phenolic antioxidants. The antioxidant potential of a range of fruit juices was assessed by measurement of their ability to reduce a synthetic free radical, potassium nitrosodisulphonate, and also by their ability to reduce Fe(III). Vitamin C was found to account for 65–100% of the antioxidant potential of beverages derived from citrus fruit but less than 5% of apple and pineapple juice. The contribution of carotenoids to antioxidant potential was negligible. Although phenolics appear to be major contributors to the antioxidant potential of the non-citrus juices, their identity and bio-availability requires further investigation.

Keywords: Antioxidant capacity; Fruit juices

1. Introduction

Dietary recommendations for healthy eating include the consumption of fruit juices (Williams, 1995) whose beneficial health effects are ascribed, in part, to vitamin C, a natural antioxidant which may inhibit the development of major clinical conditions including heart disease and certain cancers (Diplock, 1994). However, many fruit juices also contain phenolic compounds and carotenoids (Hertog, Hollman & van de Putte, 1993; Reeder & Park, 1975), some of which have antioxidant potential and whose intakes have also been inversely associated with heart disease and cancers (Hertog, Kromhout, Aravanis, Blackburn, Buzina & Fidanza, 1995; Tibble, 1998). Consequently, in order to establish the relative contribution of vitamin C, carotenoids and phenolics to the antioxidant potential of various fruit juices, we have assessed, using electron spin resonance (ESR) spectroscopy, their ability to reduce a synthetic free radical species, potassium nitrosodisulphonate. Results have been compared with antioxidant capacity estimated from the ability of the juices to reduce Fe(III). The nutritional implications of the results are discussed.

2. Materials and methods

2.1. Chemicals and reagents

Cartons of pure juices (Florida Orange, Pineapple, Jaffa Orange, Apple, Orange, Grapefruit, Pink Grapefruit and Vegetable) were purchased from a local supermarket. Sodium hydroxide, glacial acetic acid, ethylenediaminetetraacetic acid disodium salt (EDTA), acetonitrile, ethanol, sodium carbonate (anhydrous) and hydrogen peroxide were from BDH Lab Supplies (Poole, UK). Hydrochloric acid, hexane and methanol were obtained from Fisher Scientific UK Ltd. All other materials were purchased from Sigma-Aldrich (Poole, UK).

2.2. Assessment of antioxidant capacity

Two methods were employed to measure the antioxidant activity of the juices. The ability of the juices to donate a hydrogen atom or electron to the synthetic free radical potassium nitrosodisulphonate (Fremy's salt) was monitored by electron spin resonance spectroscopy (ESR) (Gardner, McPhail & Duthie, 1997; Gardner, McPhail, Crozier & Duthie, 1999). A ten-fold, aqueous dilution of fruit juice (3 ml) was added to an equal volume of Fremy's salt radical (1 mM, in 10 mM phosphate

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buffered saline, pH 7.4). The spectrum of the low field resonance of the Fremy's radical was recorded after 5 min by ESR. Signal intensity was obtained by double integration and the concentration calculated by comparison with a control reaction with distilled water instead of fruit juice. Spectra were obtained at 21°C on a Bruker ECS 106 spectrometer working at ca 9.5 GHz (X-band frequency) and equipped with a cylindrical (TM_{110} mode) cavity. The microwave power and modulation amplitude were set at 2 mW and 0.01 mT, respectively. Antioxidant capacity was expressed as the number of Fremy's radicals reduced by the juices.

Antioxidant potential of the juices was also estimated from their ability to reduce Fe(III)-2,4,6-Tri(2-pyridyl)-s-triazine (TPTZ) complex to Fe(II)-TPTZ, the resulting intense blue colour being linearly related to the amount of reductant (antioxidant) present (Benzie & Straine, 1996). The absorbance at 593 nm was measured 4 min after 1 ml of a ten-fold dilution of the juices was added to 3 ml of Fe(III)-TPTZ and ferric reducing antioxidant potential (FRAP value) was interpolated from a standard curve prepared from a stock solution.

To allow estimation of the relative contribution of the major antioxidant components to the overall antioxidant capacity of the juices, the ability of vitamin C, and β-carotene to reduce Fremy's radical was also measured using identical procedures (Gardner et al., 1997).

2.3. Determination of ascorbic acid, total phenol and carotenoid contents of the juices

Ascorbic acid contents were measured by reverse phase HPLC, adapted from the method of Ross (1994). Fresh juice samples were centrifuged at 2000×*g* (4°C, 5 min) and aliquots (0.5 ml) of supernatant were added to a similar volume of 10% meta-phosphoric acid (MPA) (0.5 ml). After vortex mixing (5 min) and centrifugation (9000×*g*, 4°C, 10 min), the clear supernatant was applied to a reverse phase HPLC (Gilson models 802/302/232, Anachem, Beds. UK) with UV detection. System conditions were: injection volume 20 μl, detector wavelength 248 nm; flow rate 1 ml/min; column Nucleosil ODS 55 mm, 25 cm×4.6 mm ID (Jones Chromatography, Glamorgan, UK), guard column pellicular C18 reverse phase 38–40 μm packing (Anachem). The mobile phase was 25 mM myristyltrimethylammonium bromide, 0.05 M sodium hydroxide, 0.06 M acetic acid, 7.5% (v/v) acetonitrile, pH 5.5. Homocysteine at 100 mg/ml and EDTA at 200 mg/ml were added before use.

The total phenol contents of the fruit juices were determined using the Folin-Ciocalteu method (Singleton & Rossi, 1965), reading samples on a Unicam UV/Vis spectrophotometer at 765 nm. Juices were centrifuged at 2000×*g* (4°C, 5 min) and diluted by a factor 10 with distilled water. Results were expressed as gallic acid equivalents (mg l^{-1}).

Measurements of total carotenoid concentrations were based on Hess et al. (1991). In brief, ethanolic solutions of the juices (300 μl juice + 300 μl distilled water + 600 μl EtOH) were extracted into hexane (1200 μl) and following centrifugation (5 min, 2000×*g*, 4°C) absorbance was scanned between 350 and 520 nm and peak areas compared to β-carotene standards. Results were calculated as β-carotene equivalents.

3. Results

The carotenoid concentrations of the juices were low and in some cases below the limits of detection (i.e. grapefruit, pineapple and apple). However, all juices contained phenolics (range 293–755 μg/ml) and the juices derived from citrus fruits were particularly rich in vitamin C. In contrast apple, pineapple and vegetable juice contained little of the vitamin (Table 1). All juices had some ability to reduce both Fremy's radical and to reduce Fe(III) to Fe(II) (Fig. 1). The two assays gave comparable results that were strongly correlated ($r = 0.96$, $P < 0.001$) although lack of juices with intermediate vitamin C concentrations may confound accurate determination of confidence intervals. However, antioxidant capacities of the different juices varied

Table 1
. Vitamin C, total phenols and total carotenoids contents of fruit juices

Juice	Vitamin C (μM)	Total phenols (μg ml^{-1} of gallic acid equivalents)	Total carotenoids (μg ml^{-1} of β-carotene equivalents)
Orange	1233 ± 36	755 ± 18	3.0 ± 1.4
Jaffa orange	1385 ± 36	591 ± 8	3.0 ± 1.1
Grapefruit	1076 ± 61	535 ± 11	nd[a]
Pink grapefruit	920 ± 18	537 ± 15	8.3 ± 2.0
Florida orange	1008 ± 66	504 ± 10	nd
Apple	3.9 ± 0.5	339 ± 43	nd
Pineapple	4.4 ± 0.5	358 ± 3	nd
Vegetable	13 ± 5	293 ± 5	8.2 ± 0.7

[a] nd, not detectable. Results as mean ± SEM of triplicate measurements.

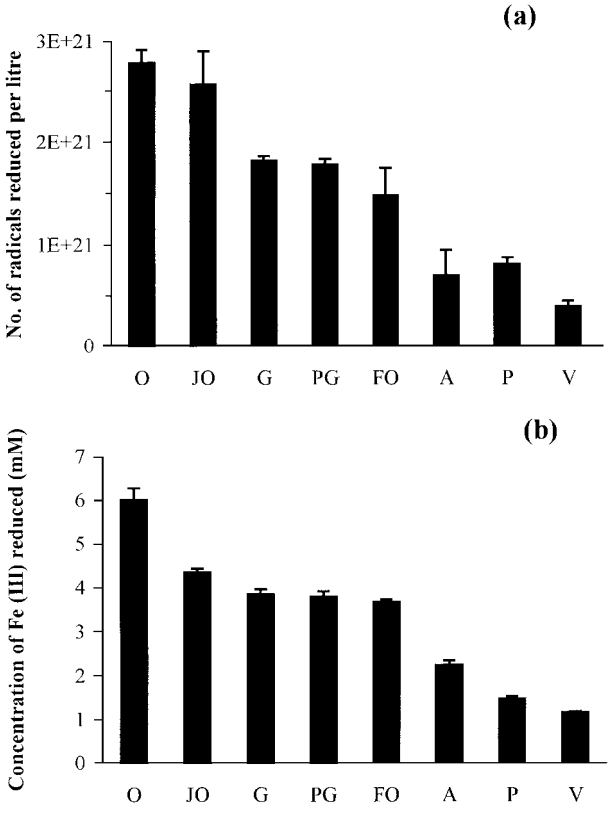

Fig. 1. Antioxidant capacities of fruit juices assessed by (a) ESR spectroscopic detection, and (b) Fe(III) reduction. The correlation between the two assays is highly significant ($r = 0.96$, $P < 0.001$). Key: O, Orange; JO, Jaffa orange; G, Grapefruit; PG, Pink grapefruit; FO, Florida orange; A, Apple; P, pineapple; V, Vegetable.

Table 2.
Correlation of electron spin resonance spectrometric (ESR) and Fe(III) reducing measurements (FRAP) of antioxidant capacity with vitamin C, phenols and carotenoids contents of the juices

	Vitamin C	Phenols	Carotenoids
ESR	0.93[a]	0.97[a]	−0.03
FRAP	0.90[a]	0.99[a]	−0.05

[a] $P > 0.001$.

markedly, orange juice being 5–7 fold more active than the vegetable juice (Fig. 1).

Both vitamin C concentrations and total phenol contents strongly correlated with antioxidant capacity as determined by both ESR and the reduction of Fe(III). No similar association was found for carotenoids (Table 2). With an identical ESR procedure, ascorbate solution reduced 2.48 Fremy's radicals per molecule. Using this figure to calculate the contribution of vitamin C to the total antioxidant capacities of the juices indicated that it accounted for 100% of antioxidant activity of Florida Orange. However, less than 5% of the antioxidant activity of apple, pineapple and vegetable juice could be ascribed to vitamin C (Table 3). As β-carotene was found to be unreactive with Fremy's radical and Fe(III), the contribution of the low concentrations of carotenoids detected to the overall antioxidant capacity of the juice was assumed to be negligible.

Table 3
Percentage contribution of vitamin C to the antioxidant capacity of the fruit juices[a]

Juice	Contribution (%)
Orange	66 ± 3
Jaffa orange	81 ± 1
Grapefruit	89 ± 4
Pink grapefruit	77 ± 3
Florida orange	100 ± 1
Apple	0.8 ± 0.2
Pineapple	0.8 ± 0.1
Vegetable	4.8 ± 1.6

[a] Mean ± SEM of triplicate determinations. Calculated from the relationship that 1 molecule of vitamin C reduces 2.48 Fremy's radicals.

4. Discussion

The ability of the fruit juices to reduce Fremy's radicals or Fe(III) was closely related to their total phenol contents and reflects the ability of many phenolic compounds to donate hydrogen atoms from hydroxyl groups on their ring structures (Scott, 1997). Similar antioxidant activity has been described for phenolic-rich beverages such as wines and teas (Gardner et al, 1997, 1999; Rice-Evans, Miller & Paganga, 1996) and has lead to suggestions that some phenolic compounds may prevent oxidative damage in vivo and thus protect against the development of diseases such as heart disease and cancer (Wiseman, 1999). Candidate phenolic antioxidants in foods include flavonoids, anthocyanins, catechins, chalcones and hydroxybenzoic and hydroxycinnamic acids many of which are present in fruit juices (Hertog et al., 1993; Kefford & Chandler, 1970).

Although all the juices used in the present study contained phenolic compounds, antioxidant activity was greatest in those which contained the highest concentrations of vitamin C. In addition, ascorbic acid accounted for 65–100% of the total antioxidant capacities of the five juices derived from citrus fruits but for less than 5% of that of the least reducing, apple, pineapple and vegetable juices. This supports the observation that ascorbic acid is the major antioxidant in orange juice but is not a major contributor to antioxidant capacity in apple juice (Rice-Evans & Miller, 1996).

It is not clear which phenolic compounds are the major contributors to the antioxidant activity of apple, pineapple and vegetable juice. Much of the antioxidant potential of teas are ascribed to catechin-derivatives

which are able to effectively reduce Fremy radical (Gardner et al., 1997). For example, (±)-catechin, epicatechin, epigallocatechin and their associated gallates reduce between 2.6 and 4.3 radicals/molecule and are therefore more effective antioxidants than vitamin C in this system (Gardner et al., 1997) and are similar in effectiveness to Trolox, a water soluble analogue of vitamin E (2.6 radicals/molecule). The Fremy's reducing ability of some other phenolics which may be present in these juices are: quercetin (1.2), rutin (0.9), myricetin (2.2) and myricitrin (1.9) (Gardner et al, 1999).

The nutritional relevance of such phenolics is uncertain as they may be poorly absorbed and rapidly metabolised and thus have limited antioxidant ability in vivo (Duthie, 1999). In contrast, vitamin C is highly bioavailable and is consequently one of the most important water-soluble antioxidants in cells, efficiently scavenging reactive oxygen species such as O_2^-, $OH^\cdot$, peroxyl radicals and singlet oxygen (Halliwell, 1996). Moreover, by efficiently trapping peroxyl radicals in the aqueous phase of the plasma or cytosol, vitamin C can protect biomembranes and low density lipoproteins from peroxidative damage (Sies, Stahl & Sundquist, 1992). Consequently, when relating the antioxidant activities of fruit juices to disease risk and health (Williams, 1995), it is important to consider the contribution of vitamin C in addition to that of phenolic compounds with antioxidant activity in chemical systems.

Both methods of assessing antioxidant potential of fruit juices gave comparable results indicating that FRAP which requires no major equipment outlay may be a cost effective option under routine conditions. However, ESR spectroscopy confers several advantages over colourimetric detection. Reactions are only likely to be significant with good H-atom donors, a necessary requirement for antioxidant function in biological systems. In addition, the radicals have well-defined spectra which will allow them to be clearly resolved from other radical intermediates which may be formed during oxidation processes. The technique is very sensitive allowing detection at the sub-micromolar level. Furthermore, analysis can be undertaken on turbid, or highly-coloured solutions, a characteristic of many plant-based foods and beverages.

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