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Total antioxidant and ascorbic acid content of fresh fruits and vegetables: implications for dietary planning and food preservation

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Epidemiological evidence links high intake of ascorbic acid (AA) and other antioxidant micronutrients to health promotion. It would be useful to know the overall, or 'total' antioxidant capacity of foods, to establish the contribution of AA to this, and to assess how this information may translate into dietary intakes to meet the new US daily reference intake for AA. In this study, the total antioxidant capacity, as the ferric reducing–antioxidant power (FRAP) value, and AA content of thirty-four types of fruits and vegetables were measured using a modified version of the FRAP assay, known as FRASC. This measures AA (reduced form only) simultaneously with the FRAP value. Results covered a wide range: 880–15 940 μmol/kg fresh wet weight and <20–540 mg/kg fresh wet weight respectively, for FRAP and AA, which comprised <1–73 % and <1–59 % total antioxidant capacity of fruits and vegetables respectively. We estimate that 100 mg AA is contained in one orange, a few strawberries, one kiwi fruit, 1–2 slices of pineapple, several florets of raw cauliflower or a handful of uncooked spinach leaves. Apples, bananas, pears and plums, the most commonly consumed fruits in the UK, contain very little AA. Results indicate also that the antioxidant capacity of vegetables decreases rapidly and significantly after fragmentation. Results of this, and future studies, using FRASC as a biomonitoring tool will be useful in food production, preparation, preservation, and aid dietary choices to increase antioxidant and AA intake. Furthermore, FRASC will facilitate bioavailability studies of antioxidants from different foods of known antioxidant capacity and AA content.

Antioxidant: Ascorbic acid: Fruit: Vegetable: Bioavailability: Food composition

Diets rich in fresh fruits and vegetables are protective against chronic, degenerative disease (Joshipura *et al.* 1999; Lampe, 1999; Cox *et al.* 2000). The beneficial effects of fruits and vegetables are hypothesised to be owing, at least in part, to antioxidants (Halliwell *et al.* 1995; Collins, 1999; Strain & Benzie, 1999). Ascorbic acid (AA) is a water-soluble antioxidant known to be important to health (Benzie, 1999; Davey *et al.* 2000). This has been recognised in the recent increase in the US daily reference intake of AA to 75 and 90 mg/d respectively, for women and men, with an additional 35 mg/d recommended for smokers (Larkin, 2000). Plant-based food items contain a wealth of phytochemicals, however, and many have antioxidant properties (Halliwell *et al.* 1995; Hollman & Katan, 1997; Strain & Benzie, 1999; Duthie *et al.* 2000). In food analysis, individual phytochemicals can be identified and measured, and the comprehensive characterisation of the antioxidant profile of fruits and vegetables is important. However, it would be useful to know the overall, or 'total' antioxidant power (Benzie & Strain, 1996) of foods, to establish the contribution of AA to this, and to assess how this information may translate into dietary intakes to meet the new US daily reference intake for vitamin C. In the present study, the total antioxidant capacity, as the ferric reducing–antioxidant power (FRAP) value, and the AA content of a range of fresh fruits and vegetables were measured. We used a sensitive biomonitoring tool referred to as FRASC (ferric reducing–antioxidant power and ascorbic acid; Benzie & Strain, 1997). This is a modified version of the FRAP assay (Benzie & Strain, 1996; US patented) which measures AA simultaneously with the FRAP value. The FRAP value and the relative and absolute contributions of AA to the total antioxidant capacity of the fruits and vegetables tested are presented.

Abbreviations: AA, ascorbic acid; FRAP, ferric reducing–antioxidant power; FRASC, ferric reducing–antioxidant power and ascorbic acid.

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Materials and methods

Extracts of washed, uncooked, fresh fruits and vegetables were prepared by homogenising 5 g (fresh wet weight of the edible part, with or without skin and seeds, as would normally be eaten) in 100 ml distilled water for 30 s. Homogenates were filtered, and the total antioxidant capacity and AA (reduced form only) concentrations were measured in triplicate immediately afterwards using an assay known as FRASC (Benzie & Strain, 1997). Water extracts of vegetables had a near neutral pH, therefore extracts in acetate buffer (pH 3·6) were also made, as AA is unstable at neutral pH. We compared FRAP and AA values of neutral pH and acid extracts to determine if there was a significant, rapid loss of antioxidants in uncooked vegetables following cellular disruption. Extracts of fruits were acidic, and only water extracts of these were made. Seventeen varieties of fruits and seventeen different types of vegetables were tested. For each fruit and vegetable at least three samples, purchased from local shops, were tested, each in triplicate. Results presented are the total antioxidant content as the FRAP value (as μmol/kg wet weight), the AA content (as μmol/kg wet weight, and as mg/kg wet weight), and the % total antioxidant capacity of the extract contributed by AA.

The FRASC assay was performed as described in detail elsewhere (Benzie & Strain, 1997, 1999). In brief, reductants ('antioxidants') in the sample reduce a ferric–tripyridyltriazine complex, present in stoichiometric excess, to the blue coloured ferrous form. The change of absorbance at 593 nm over 4 min is proportional to the combined (total) FRAP value of the antioxidants in the sample. In the FRASC assay, AA is selectively destroyed by the addition of ascorbate oxidase to one of a pair of sample aliquots. In this case the absorbance change of a sample to which ascorbate oxidase was added is subtracted from the absorbance of a matching aliquot to which water, rather than ascorbate oxidase was added; the difference is due specifically to AA (reduced form only). Change in absorbance (0–1 min for AA and 0–4 min for FRAP) is converted to μmol/l by comparison with a standard of known AA concentration or FRAP value. Aqueous solutions of ferrous ions (iron(II) sulfate ($FeSO_4.7H_2O$)) and ascorbic acid (L(+)-AA, extra pure crystals) both from Merck (Darmstadt, Germany) are used as calibrators. The FRAP assay has a limit of detection of 2 μmol/l, and precision is excellent: within- and between-run CV are <0·5 and 1·0 % respectively at between 500 and 2000 μmol/l antioxidant–reducing power, $n>8$ in each case. For AA, within- and between-run CV of FRASC are <5 % at 25, 50, 100, and 440 μmol/l.

All reagents and solutions were prepared in Milli-Q water, which was made from a Millipore ultrapure water system (Millipore Corp., Bedford, MA, USA). FRASC reagents were as follows: 300 mmol/l acetate buffer, pH 3·6, prepared by dissolving 3·1 g sodium acetate trihydrate in distilled water, with 16 ml glacial acetic acid (BDH Laboratory Supplies, Poole, UK) added and made up to 1 litre with distilled water; 10 mM-2,4,6 tripyridyl-*S*-triazine (Fluka Chemicals, Buchs, Switzerland) solution in 40 mM-HCl (BDH Laboratory Supplies); 20 mM-$FeCl_3.6H_2O$ (BDH Laboratory Supplies) solution in distilled water. Working FRASC reagent was prepared as needed by mixing 10 ml acetate buffer with 1 ml 2,4,6 tripyridyl-*S*-triazine solution and 1 ml $FeCl_3.6H_2O$ solution. A 4 IU ascorbic oxidase/ml solution (Sigma Chemical Co., St, Louis, MO, USA) was prepared in distilled water, divided into portions and stored at −70°C until needed.

For FRASC analysis, 100 μl of each freshly prepared food extract was mixed with 40 μl ascorbic oxidase solution; a matching (paired) 100 μl aliquot of each extract was mixed with 40 μl water; paired extracts were immediately loaded on the analyser (Cobas Fara centrifugal analyser; Roche Diagnostics Ltd, Basel, Switzerland). The 0–1 min change in A_{593} nm readings of the paired extracts (tested in parallel) were retrieved and used to calculate the AA concentration. The 0–4 min changes in A_{593} nm of the extracts treated with water only (no ascorbic oxidase) were retrieved and used to calculate the FRAP values. The FRAP value in μmol/l was calculated by simple comparison of 0–4 min change in absorbance at 593 nm of the test sample and that of a Fe^{2+} calibrator, as follows:

$$\frac{\text{0–4 min } \Delta A_{593\,\text{nm}} \text{ of test sample}}{\text{0–4 min } \Delta A_{593\,\text{nm}} \text{ of standard}} \times [Fe^{2+}] \text{ standard } (\mu\text{mol/l}).$$

The AA value in μmol/l was calculated by comparison of 0–1 min change in absorbance at 593 nm of the extract and that of an AA calibrator, as follows:

$$\text{0–1 min } \Delta A_{593\,\text{nm}} = (\text{0–1 min } \Delta A_{593\,\text{nm}} \text{ sample} - \text{ao}) - (\text{0–1 min } \Delta A_{593\,\text{nm}} \text{ sample} + \text{ao}),$$

and

$$\text{AA concentration } (\mu\text{mol/l}) = \frac{\text{0–1 min } \Delta A_{593\,\text{nm}} \text{ of test sample}}{\text{0–1 min } \Delta A_{593\,\text{nm}} \text{ of standard}} \times [\text{AA}] \text{ standard } (\mu\text{mol/l}),$$

where ao is ascorbic oxidase.

AA was converted from μmol/l extract to mg/kg wet weight of fruit or vegetable by simple calculation based on molecular mass of AA (176), the weight of fruit or vegetable extracted and the volume of fluid used for extraction (typically 5 g and 100 ml respectively). The % contribution of AA (which has a stoichiometric value of 2 in the FRAP assay, i.e. 1 mol AA reduces 2 mol Fe^{3+} to Fe^{2+}) to the total antioxidant capacity (FRAP value) of each fruit or vegetable tested was calculated as follows:

$$\frac{\text{AA } (\mu\text{mol/l}) \times 2}{\text{FRAP value } (\mu\text{mol/l})} \times 100\,\%.$$

Results

Results for FRAP and AA showed a wide range (Tables 1 and 2). AA comprised <1–73 % total antioxidant content of fruits, and <1–59 % for vegetables. Strawberries were

Table 1. Total antioxidant power* and ascorbic acid content of aqueous extracts of fruits†
(Mean values and standard deviations for triplicate determinations on three or more fruits)

Fruit	FRAP (μmol/kg fresh wet wt) Mean	FRAP SD	Ascorbic acid (mg/kg fresh wet wt) Mean	Ascorbic acid SD	'Total' vitamin C (ascorbic acid plus dehydroascorbic acid) (mg/kg fresh wet wt)‡	Contribution (%) of ascorbic acid to total antioxidant power§	Tested with (+) or without (−) skin and/or seed
Strawberry	15940	2780	540	60	770	39	+/+
Lemon	10400	1480	420	70	580	46	−/−
Plum	9280	2240	10	10	40	<1	+/−
Orange	9420	2740	330	40	540	40	−/−
Kiwi fruit	8200	220	520	100	590	73	−/+
Grapefruit	8080	300	390	10	360	54	−/−
Persimmon	7740	5020	80	60		11	+/−
Apple (green)	6300	160	<50		60	<1	+/−
Apple (red)	4200	420	<10			<1	+/−
Mandarin	5400	760	240	20	200‖	50	−/−
Mango	5060	140	210	40	370	48	−/−
Grape (green)	4780	2300	20	10	30	6	+/−
Grape (red)	4160	980	<10			<1	+/−
Banana	4200	320	<10		110	<1	−/−
Pear	4080	1080	<10		60	<1	+/−
Pineapple	3480	1420	100	20	120	33	−/−
Chinese pear	1460	440	<10			<1	+/−

FRAP, ferric reducing–antioxidant power.
* Expressed as the FRAP value.
† For details of samples and procedures, see p. 56.
‡ Values taken from *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1991).
§ The stoichiometric factor of ascorbic acid in the FRAP assay is 2·0; hence, 1 μmol ascorbic acid is equivalent to 2 μmol antioxidant power (as FRAP).
‖ Value is for canned mandarins.

particularly high in AA and antioxidant capacity. Kiwi fruit contained the highest proportion of AA. Several fruits, however, contained negligible amounts of AA. Of the vegetables tested, choy sum (a Chinese green leafy vegetable similar to kale) had the highest antioxidant capacity. Iceberg lettuce was lowest in antioxidant capacity. There was significant AA in cauliflower, in several Chinese vegetables (choy sum, pak choy, wombok (a cabbage-like Chinese vegetable)), and in spring onion. Results on acidic extracts of some vegetables (cauliflower, cabbage, spring onion and broccoli) were markedly higher than those of water extracts, even though both water- and acetate buffer-extracts were made in parallel and measured within a few minutes of fragmentation (Table 2).

Discussion

Issues of bioavailability mean that caution is needed when interpreting results of *in vitro* measures, as have been performed here, in an *in vivo* context. Nonetheless, knowing the AA content of individual foods is useful in planning dietary strategies to meet the new US reference daily intake for AA. In addition, the total antioxidant capacity and the relative contribution to this of AA and non-AA components may be useful indices of the potential health benefits of individual dietary agents. Furthermore, measurement of total antioxidant capacity could be a valuable tool in food technology, as the effect of growing conditions, seasonality, storage, processing, preservation techniques, cooking and genetic modification of plant-based foods could be determined.

The results presented here show that the total antioxidant capacity of fresh fruits and vegetables varies widely, indicating clearly that all servings are not equal in terms of antioxidant intake. Strawberries have an antioxidant capacity up to 10-fold greater than that of other fruits. Plums, oranges, kiwi fruit and grapefruit also have high antioxidant capacity. However, our results show also that plums, while high in other antioxidants, are low in AA. Several other fruits are also low in AA, including apples, pears, grapes and bananas. Evidently, the most commonly consumed fruits in UK are the ones that have the lowest vitamin C content. Adequate intake, however, could be achieved (in theory) with just one serving of an AA-rich fruit or vegetable. We estimate that about 100 mg vitamin C is contained within one medium-sized orange, two large strawberries, one kiwi fruit, two slices of pineapple, or one small grapefruit. A few florets of raw cauliflower or a handful of uncooked kale or spinach leaves also contain enough vitamin C to meet the revised US reference daily intake. However, bioavailability of AA and other antioxidants from different food sources is not completely known, and further work is needed in this area.

Comparing our AA data with published values (taken from *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1991), shown in Tables 1 and 2), it can be seen that the results of our water extracts were lower, and for some vegetables (mange tout and broccoli in particular) markedly so. In Hong Kong these vegetables are invariably imported from overseas, and sub-optimal transport and storage conditions may have resulted in a considerable loss of AA, and perhaps other antioxidants, before purchase of these 'fresh' vegetables. It is unlikely that the method used in this current study (FRASC) underestimated the AA

Table 2. Total antioxidant power* and ascorbic acid content of vegetables extracted with water and with acetate buffer (pH 3·6)† (Mean values and standard deviations for triplicate determinations on three or more vegetables)

	FRAP (μmol/kg fresh wet wt)				Ascorbic acid (mg/kg fresh wet wt)						
	In water		In acetate buffer		In water		In acetate buffer		'Total' vitamin C (ascorbic acid plus dehydroascorbic acid) (mg/kg fresh wet wt)‡	Contribution (%) of ascorbic acid to total antioxidant power§	
Vegetable	Mean	SD	Mean	SD	Mean	SD	Mean	SD		In water	In acetate buffer
Choy sum	10420	2820	10440	2040	130	60	29	14		14	31
Pak choy	6200	900	8440	480	90	20	28	11		17	38
Spring onion	5900	540	8040	1440	150	20	28	5	260	30	39
Wombok	5620	2760	6800	4200	200	80	28	17		40	46
Mange-tout	4900	960	4460	1240	<10		11	1	540	<1	29
Onion	4320	1260	2880	1300	80	60	5	5	50	22	19
Turnip (green)	3580	2160	3720	2500	140	110	15	10	170	44	45
Cabbage (long)	3500	720	5000	1120	110	40	15	6	490	34	34
Broccoli	2940	560	7480	2460	10	20	10	4	870	4	14
Cauliflower	2840	640	5880	2420	140	80	30	23	430	56	59
Garlic	2680	1000	2400	380	60	50	5	2	170	27	22
Tomato	2360	140	3120	580	120	60	9	4	170	58	32
Chinese lettuce	2300	460	2280	1440	<10		1	1		<1	3
Carrot	1660	800	2400	1500	10	10	1	1	60	5	7
Celery	1560	60	1340	440	<10		3	1	80	<1	27
Potato	1440	320	2320	960	<10		5	2	70	<1	26
Lettuce (iceberg)	880	260	580	160	<10		<1		30	<1	<1

FRAP, ferric reducing–antioxidant power.
* Expressed as the FRAP value.
† For details of samples and procedures, see p. 56.
‡ Values taken from *McCance and Widdowson's The Composition of Foods* (Holland *et al.* 1991).
§ The stoichiometric factor of ascorbic acid in the FRAP assay is 2·0; hence, 1 μmol ascorbic acid is equivalent to 2 μmol antioxidant power (as FRAP).

content *per se*, as we have shown this method to be specific, accurate and highly reproducible (Benzie & Strain, 1997, 1999) and AA results obtained by FRASC are very similar to those obtained by a reference HPLC method (WY Chung, YT Szeto and IFF Benzie, unpublished results). In addition, we used freshly prepared extracts, thus minimising post-extraction, pre-analytical loss. It should be noted, however, that the previously published AA results (Holland *et al.* 1991) presented in Tables 1 and 2 represent the sum of the reduced plus oxidised forms of AA, while our results, using FRASC, are for reduced AA only. This methodological difference helps explain at least some of the difference seen. This suggestion is supported by the finding that AA (reduced form) was higher in acidic extracts of most vegetables, indicating that some AA in the water extracts had been oxidised to dehydroascorbic acid before measurement. Nonetheless, some of the difference may be owing to differences in seasonality or variety tested, or related to geographical factors, and this requires further study. We suggest that the simplicity and speed of the FRASC assay makes this a very attractive method for performing studies of this type.

This current study did not investigate the effect of cooking or storage on antioxidant content, but our results indicate that loss of antioxidants from chopped, shredded or pureed vegetables may be rapid and significant. Polyphenol oxidase is found in many plants (Martinez & Whitaker, 1995; Das *et al.* 1997), and is responsible for the browning seen in bruised, fragmented or chopped fruit, and for the change, from green to black, which occurs in crushed or chopped tea leaves. The antioxidant capacity of green tea is much higher than that of black tea (Benzie & Szeto, 1999) because of enzymatic oxidation of polyphenolic tea antioxidants (Weisburger, 1996). A similar loss of phenolic-related antioxidant power in vegetables is likely to occur with crushing, chopping or pureeing. Interestingly, disruption of the vegetable matrix has been reported to increase the bioavailability of folate and lutein, but not of β-carotene or AA (van het Hof, 1999). Our results suggest that, following cellular disruption, less AA and other antioxidants than expected may remain in the food matrix, and this may affect their apparent bioavailability. A further use of FRASC as a biomonitoring tool, therefore, is in determining the bioavailability of antioxidants in food by monitoring changes in plasma AA concentration and FRAP value after ingestion of food of known (measured) AA and FRAP content.

In conclusion, this current study presents new data, using a novel approach, on the antioxidant capacity and the absolute and relative content of reduced AA in a range of fresh fruits and vegetables, including some Chinese varieties not previously studied. The method used, the FRASC assay, is rapid, reproducible, and relatively simple, making it an attractive biomonitoring tool for nutrition and food technology studies. Results indicate that, in terms of antioxidant capacity and AA, a single serving of some fruits and vegetables is worth several servings of others. Furthermore, results show a rapid loss of antioxidants following fragmentation of some vegetables, and this is prevented by mild acidification. While we do not yet know if increased antioxidant intake is directly beneficial to human health, there is nevertheless a strong inverse

relationship between dietary antioxidants and all cause mortality, as reported most recently by Khaw *et al.* (2001). Until the active component(s) of fruits and vegetables are clearly established, measuring their total antioxidant and AA content may be useful in planning diets for health promotion. Furthermore, if it is confirmed that the health benefits of fruits and vegetables are mediated through their antioxidant content, optimising antioxidant intake will become a primary aim in preventive medicine, and determining bioavailability of antioxidants from different food sources will become a priority. Food tables will require updating to include the antioxidant capacity and profile of foods, as well as data on tropical, oriental, and genetically modified varieties not previously studied. FRASC is suitable for this type of work, and will help provide objective data to help plan diets with high antioxidant content and bioavailability. Furthermore, FRASC is useful for studies evaluating methods of food production, preservation, preparation and storage in terms of effects on antioxidant capacity and AA content.

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