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 mmol/l) and 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 (FeSO₄.7H₂O)) 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 10% 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 31.2 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-FeCl₃·6-H₂O (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 FeCl₃·6H₂O 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 acid 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 A₅₉₃ nm readings of the paired extracts (tested in parallel) were retrieved and used to calculate the AA concentration. The 0–4 min changes in A₅₉₃ 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 Fe²⁺ calibrator, as follows:

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

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:

\[
0–1 \text{ min } \Delta A_{593 \text{ nm}} = (0–1 \text{ min } \Delta A_{593 \text{ nm}} \text{ sample} – a_o)
- (0–1 \text{ min } \Delta A_{593 \text{ nm}} \text{ sample} + a_o),
\]

and

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

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 Fe³⁺ to Fe²⁺) to the total antioxidant capacity (FRAP value) of each fruit or vegetable tested was calculated as follows:

\[
\text{AA \ (μmol/l) \times } \frac{2}{\text{FRAP value \ (μmol/l)}} \times 100 \%.
\]

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)

<table>
<thead>
<tr>
<th>Fruit</th>
<th>FRAP (μmol/kg fresh wet wt)</th>
<th>Ascorbic acid (mg/kg fresh wet wt)</th>
<th>‘Total’ vitamin C (ascorbic acid plus dehydroascorbic acid) (mg/kg fresh wet wt)‡</th>
<th>Contribution (%) of ascorbic acid to total antioxidant power§</th>
<th>Tested with (-) or without (+) skin and/or seed</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strawberry</td>
<td>15940</td>
<td>540</td>
<td>770</td>
<td>39</td>
<td>+/+</td>
</tr>
<tr>
<td>Lemon</td>
<td>10400</td>
<td>420</td>
<td>580</td>
<td>46</td>
<td>-/‐</td>
</tr>
<tr>
<td>Plum</td>
<td>9280</td>
<td>10</td>
<td>40</td>
<td>&lt;1</td>
<td>+/‐</td>
</tr>
<tr>
<td>Orange</td>
<td>9420</td>
<td>330</td>
<td>540</td>
<td>40</td>
<td>-/‐</td>
</tr>
<tr>
<td>Kiwi fruit</td>
<td>8200</td>
<td>520</td>
<td>590</td>
<td>73</td>
<td>-/+</td>
</tr>
<tr>
<td>Grapefruit</td>
<td>8080</td>
<td>390</td>
<td>360</td>
<td>54</td>
<td>-/‐</td>
</tr>
<tr>
<td>Persimmon</td>
<td>7740</td>
<td>80</td>
<td>11</td>
<td>11</td>
<td>+/‐</td>
</tr>
<tr>
<td>Apple (green)</td>
<td>6300</td>
<td>&lt;50</td>
<td>60</td>
<td>&lt;1</td>
<td>+/‐</td>
</tr>
<tr>
<td>Apple (red)</td>
<td>4200</td>
<td>&lt;10</td>
<td>&lt;1</td>
<td>1/‐</td>
<td>+/‐</td>
</tr>
<tr>
<td>Mandarin</td>
<td>5400</td>
<td>240</td>
<td>200</td>
<td>50</td>
<td>-/‐</td>
</tr>
<tr>
<td>Mango</td>
<td>5060</td>
<td>210</td>
<td>370</td>
<td>48</td>
<td>-/‐</td>
</tr>
<tr>
<td>Grape (green)</td>
<td>4780</td>
<td>20</td>
<td>30</td>
<td>6</td>
<td>+/-</td>
</tr>
<tr>
<td>Grape (red)</td>
<td>4160</td>
<td>&lt;10</td>
<td>110</td>
<td>&lt;1</td>
<td>+/‐</td>
</tr>
<tr>
<td>Banana</td>
<td>4200</td>
<td>&lt;10</td>
<td>60</td>
<td>-/‐</td>
<td>+/‐</td>
</tr>
<tr>
<td>Pear</td>
<td>4080</td>
<td>&lt;10</td>
<td>&lt;1</td>
<td>1/‐</td>
<td>+/‐</td>
</tr>
<tr>
<td>Pineapple</td>
<td>3480</td>
<td>100</td>
<td>120</td>
<td>33</td>
<td>-/‐</td>
</tr>
<tr>
<td>Chinese pear</td>
<td>1460</td>
<td>&lt;10</td>
<td>&lt;1</td>
<td>1/‐</td>
<td>+/‐</td>
</tr>
</tbody>
</table>

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; 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...
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 | 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 | 110 | 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

Table 2. Total antioxidant power* and ascorbic acid content of vegetables extracted with water and with acetate buffer (pH 3.6)†

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>FRAP (μmol/kg fresh wet wt)</th>
<th>Ascorbic acid (mg/kg fresh wet wt)</th>
<th>‘Total’ vitamin C (ascorbic acid plus dehydroascorbic acid) (mg/kg fresh wet wt)‡</th>
<th>Contribution of ascorbic acid to total antioxidant power§</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>In water</td>
<td>In acetate buffer</td>
<td>In water</td>
<td>In acetate buffer</td>
</tr>
<tr>
<td>Choy sum</td>
<td>10420</td>
<td>2820</td>
<td>10440</td>
<td>2040</td>
</tr>
<tr>
<td>Pak choy</td>
<td>6200</td>
<td>900</td>
<td>8440</td>
<td>480</td>
</tr>
<tr>
<td>Spring onion</td>
<td>5900</td>
<td>540</td>
<td>8040</td>
<td>1440</td>
</tr>
<tr>
<td>Wombok</td>
<td>5620</td>
<td>2760</td>
<td>6800</td>
<td>4200</td>
</tr>
<tr>
<td>Mange-tout</td>
<td>4900</td>
<td>960</td>
<td>4460</td>
<td>1240</td>
</tr>
<tr>
<td>Onion</td>
<td>4320</td>
<td>1260</td>
<td>2880</td>
<td>1300</td>
</tr>
<tr>
<td>Turnip (green)</td>
<td>3580</td>
<td>2160</td>
<td>3720</td>
<td>2500</td>
</tr>
<tr>
<td>Cabbage (long)</td>
<td>3500</td>
<td>720</td>
<td>5000</td>
<td>1120</td>
</tr>
<tr>
<td>Broccoli</td>
<td>2940</td>
<td>560</td>
<td>7480</td>
<td>2460</td>
</tr>
<tr>
<td>Cauliflower</td>
<td>2840</td>
<td>640</td>
<td>5880</td>
<td>2420</td>
</tr>
<tr>
<td>Garlic</td>
<td>2680</td>
<td>1000</td>
<td>2400</td>
<td>380</td>
</tr>
<tr>
<td>Tomato</td>
<td>2360</td>
<td>140</td>
<td>3120</td>
<td>580</td>
</tr>
<tr>
<td>Chinese lettuce</td>
<td>2300</td>
<td>460</td>
<td>2280</td>
<td>1440</td>
</tr>
<tr>
<td>Carrot</td>
<td>1660</td>
<td>800</td>
<td>2400</td>
<td>1500</td>
</tr>
<tr>
<td>Celery</td>
<td>1560</td>
<td>60</td>
<td>1340</td>
<td>440</td>
</tr>
<tr>
<td>Potato</td>
<td>1440</td>
<td>320</td>
<td>2320</td>
<td>960</td>
</tr>
<tr>
<td>Lettuce (iceberg)</td>
<td>880</td>
<td>260</td>
<td>580</td>
<td>160</td>
</tr>
</tbody>
</table>

FRAP, ferric reducing–antioxidant power.

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§ The stoichiometric factor of ascorbic acid in the FRAP assay is 2; 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 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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References


