Antioxidant Properties of Fruit and Vegetable Juices:
More to the Story than Ascorbic Acid

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Abstract. Dietary supplements such as vitamin C have become popular for their perceived ability to enhance the body’s antioxidant defenses. Reactive oxygen species (ROS) have been shown to cause a broad spectrum of damage to biological systems. Scavenging of ROS is part of a healthy, well-balanced, antioxidant defense system. The present study used the Fenton reaction as a source of hydroxyl radicals and xanthine/xanthine oxidase as a source of superoxide radicals to investigate the scavenging capabilities of various fruit and vegetable juices against these radicals. Electron spin resonance (ESR) spin trapping was used for free radical detection and measurement. Using a colormetric assay, the present study also investigated the protective effects of fruit and vegetable juices against lipid peroxidation induced in cell membranes by hydroxyl radicals. The present study showed that the free radical scavenging capability of each individual juice, but not its ascorbic acid content, is correlated with its protective effect on free radical induced lipid peroxidation. The results indicate that ascorbic acid is only one facet of the protective effect of fruit and vegetable juices. It appears that consumption of whole fruits and vegetables would be superior to an ascorbic acid supplement for antioxidant effectiveness. (received 21 September 2001, accepted 8 November 2001)

Keywords: fruit and vegetable juice, antioxidants, ascorbic acid, free radicals, electron spin resonance

Introduction

Recent reports have indicated a need for increased dietary intake of ascorbic acid as a source for antioxidants [1,2]. One proposed method to increase ascorbic acid (vitamin C) intake is to ingest it as a supplemental tablet. However, studies have shown that vitamin C marketed as a dietary supplement may also act as a pro-oxidant [3] and may even react with iron to cause a cycle of hydroxyl radical (•OH) production [4]. Two laboratories have described the antioxidant capacity of fruits, in general [5], and apples, specifically [6], by measuring total antioxidant capacities.

Reactive oxygen species (ROS) have been shown to play important roles in carcinogenesis by directly damaging DNA and acting as tumor promoters [7,8]. ROS include, but are not limited to, •OH and superoxide radical (O2•−) and hydrogen peroxide (H2O2). ROS-mediated reactions are believed to be involved in various pathogenic processes [7-9]. Initiation of diseases can be associated with an imbalance caused by an excessive generation of ROS, resulting in oxidative stress.

Using electron spin resonance (ESR), the present study investigated the antioxidant properties of fruits and vegetable juices against specific radical types, •OH and O2•− radicals. The present study also determined the ability of these natural foods to protect against lipid peroxidation caused by ROS. These radicals are highly reactive and other studies have shown that they can cause cellular injury...
through various mechanisms, such as DNA damage, lipid peroxidation, and activation of the nuclear factor kappa B (NF-κB) and tumor necrosis factor alpha (TNF-α) signaling pathways [10,11]. The present study also measured the ascorbic acid contents of various natural food juices and attempted to correlate their ascorbic acid levels with their abilities to quench specific radicals and to protect cell membranes from ROS-induced damage.

Materials and Methods

Sample preparation. Several varieties of fresh fruits and vegetables were obtained from Kroger, Inc. The fruits and vegetables were washed with a mild detergent and rinsed with distilled/deionized water (dH2O) to remove possible pesticide and preservative residues. Fruit and vegetable samples were obtained by excising 100 mg of skin and pulp, crushing it, and homogenizing the sample on ice with a “tissue tearor” (Biospec Products, Racine, WI). Each sample was diluted to 1.0 ml with dH2O, homogenized again, and filtered through a nitrocellulose membrane (pore size, 0.45 µm) in order to yield the juice.

Electron spin resonance (ESR). ESR spin trapping was used to detect short-lived free radical intermediates [12]. This technique involved the addition-type reaction of a short-lived radical with a paramagnetic compound (spin trap) to form a relatively long-lived free radical product (spin adduct), which was then studied using conventional ESR. The intensity of the signal was used to measure the amount of short-lived radicals trapped. The hyperfine couplings of the spin adduct were generally characteristic of the original trapped radicals.

Spin trapping is a method of choice for detection and identification of free radical generation, owing to its specificity and sensitivity. Hyperfine couplings were measured (to 0.1 G) directly from magnetic field separation using potassium tetraperoxochromate (K2CrO8) and 1,1-diphenyl-2-picryl-hydrazyl (DPPH) as reference standards.

The relative radical concentration was estimated by multiplying half of the peak height by (ΔHpp)2, where ΔHpp represents peak-to-peak width. A SPEX 300 program (U.S. EPR, Clarksville, MD) was used for data acquisition and analyses. Both •OH and O2•− radicals were measured by ESR (Model ESP 300E, Bruker Instruments, Billerica, MA), using a flat cell assembly with 5,5-dimethyl-1-pyrroline N-oxide (DMPO) as a spin trap. Hydroxyl radicals were generated by the Fenton reaction (Fe(II) + H2O2 → Fe(III) + OH− + •OH). Superoxide radicals were generated by xanthine/xanthine oxidase. For •OH measurement, spectra were taken 3 min after reaction initiation; for O2•− measurement, the spectra were recorded 1 min after reaction initiation. All experiments were performed at room temperature in phosphate buffered saline (PBS) that had been purified by filtration through Chelex 100 resin (Sigma Chemical Co, St. Louis, MO).

Lipid peroxidation measurement. Lipid peroxidation of RAW 264.7 mouse peritoneal monocytes was measured by a colorimetric assay for lipid peroxidation products (LPO-586 kit, Oxis International Inc. Portland, OR). A typical reaction mixture contained FeSO4 (0.1 mM) + H2O2 (1 mM) and 1 x 107 cells in a total volume of 1.0 ml of PBS (pH 7.4). Fruit and vegetable juices were added (100 mg/ml) to this mixture to measure their effect on lipid peroxidation. The mixture was exposed for 1 hr in a shaking water bath at 37°C. The measurement of lipid peroxidation is based on the reaction of a chromogenic reagent with malonaldehyde and 4-hydroxyalkenals after a further incubation at 45°C for 60 min [13]. Standards and reagent blanks were used to generate a standard curve. The absorbance of the supernates was measured at 586 nm and the percent inhibition caused by the fruit and vegetable juices was calculated by comparisons to the control reaction.

Ascorbic acid measurement. L-Ascorbic acid content was measured in samples (100 mg/ml) of fruit and vegetable juices using a colorimetric measurement kit (L-Ascorbic acid reagent kit, Boehringer-Mannheim, Germany). L-Ascorbic acid was quantified from the reaction of MTT (3-(4,5-dimethylthiazolyl-2)-2,5-diphenyl-tetrazolium bromide) and PMS (5-methylphenazinium methosulfate) in the presence of L-ascorbic acid to
form the product, MTT-formazan, which has maximum absorbance at 578 nm. The analytical detection limit was 0.1 mg/L[14].

Statistical analysis. Each analytical result was based on three separate samples taken from different locations on the fruits and vegetables. The data in Table 1 were analyzed by non-paired t-test. The level of significance was set at p <0.001.

Results

Fig. 1a shows a typical ESR spectrum generated from a mixture containing the Fenton reagents in the presence of DMPO as a spin trap. This spectrum consists of a 1:2:2:1 quartet with splittings of \( a_H = a_N = 14.9 \) G. Based on these splittings constants, the 1:2:2:1 quartet was assigned to a DMPO/•OH adduct. Addition of apple juice reduced the signal intensity (Fig. 1b). Garlic juice (Fig. 1c) eliminated the •OH spin adduct signal, while green pepper juice (Fig. 1d) and orange juice (Fig. 1e) greatly reduced the signal intensity. Addition of green pepper or orange juice also caused the generation of an ascorbate radical signal indicated by (*) in Fig. 1. It may be noted that garlic juice, which completely reduced •OH radicals, did not show any observable ascorbate radical peak, suggesting that ascorbate in the garlic juice contributed very little to the •OH scavenging ability of that juice.

Table 1 displays the effectiveness of all juices as •OH scavengers. This effect on observed •OH radical peak heights ranged from 39% decrease with pear juice to 100% decrease with garlic, lemon, lime, or strawberry juices. These results show that all juices sampled have the capacity to scavenge •OH radicals, with the efficiency dependent on the type of juice.

Fig. 2 shows the effects of the same selection of juices shown in Fig. 1 on \( \text{O}_2^-\)• radicals. The spectrum recorded from a solution containing xanthine and xanthine-oxidase reaction alone is shown in Fig. 2a. With the addition of apple juice (Fig. 2b), the intensity of \( \text{O}_2^-\)• radical peaks was reduced. Garlic juice (Fig. 2c), green pepper (Fig. 2d), and orange (Fig. 2e) all reduced the \( \text{O}_2^-\)• radicals significantly. The ascorbate radical signal indicated by (*) in Fig. 2 was observed in the green pepper and orange juice samples. Garlic juice, which showed no ascorbate radical peak, was the most effective scavenger of \( \text{O}_2^-\)•. This result indicated that ascorbate did not directly contribute to the \( \text{O}_2^-\)• scavenging ability of this juice.
Table 1 displays the effectiveness of all juices as $\text{O}_2^•$ scavengers. This effect on observed $\text{O}_2^•$ radical peak heights ranged from 43% decrease with cucumber juice to 96% decrease with lemon, romaine lettuce, or strawberry juices. The results thus show that with different potencies, all tested juices function as an $\text{O}_2^•$ scavenger.

Lipid peroxidation is an indicator of possible free radical damage to cells. It has been shown that $\cdot \text{OH}$ radicals are able to cause cell membrane damage and initiate lipid peroxidation [15]. Lipid peroxidation would result in the release of lipid-derived radicals ($\text{R}^•$, $\text{RO}^•$, and $\text{ROO}^•$) [16, 17]. These reactive species could further react with the cell membrane, leading to additional damage. Injury of cell membranes may lead to increased release of catalytically active iron [18]. The products of lipid peroxidation, malondialdehyde, and other groups of aldehyde products such as hexanal, 4-hydroxylnonenal, and related aldehydes, may also cause DNA damage [19,20]. It has been proposed that free radicals derived from lipid peroxidation may function as tumor initiators [20-22].

Since previous studies [23,24] have shown that $\cdot \text{OH}$ radical was able to induce lipid peroxidation, the present study also examined the ability of natural food juices to quench the $\cdot \text{OH}$ radical and inhibit lipid peroxidation. Table 1 shows the percent inhibition provided by various natural food juices versus the amount of lipid peroxidation in control RAW 264.7 cells. Table 1 shows that all of the juices were effective inhibitors of lipid peroxidation. This effect on lipid peroxidation ranged from 31% decrease for white grape juice to a 93% decrease with garlic juice. Addition of natural food juice (100 mg/ml) to the cells before exposure to $\cdot \text{OH}$ radicals inhibited the damage to the cell membranes.

Table 1 also shows the amount of ascorbic acid present in each juice sample. These amounts range from 3.4 mg/100 g in pear juice to 125.3 mg/100 g in green pepper juice. This indicates that ascorbic acid is not the only factor that acts to scavenge free radicals. Orange juice, which contained an ascorbic acid concentration of 60 mg/100 g, reduced $\cdot \text{OH}$ and $\text{O}_2^•$ ESR peak heights by 98% and 89%, respectively, while decreasing lipid peroxidation by 77%. Garlic, which contained only 15.3 mg/100 g of ascorbic acid, reduced the $\cdot \text{OH}$ and $\text{O}_2^•$ peak heights by 100% and 94%, respectively, and lipid peroxidation by 93%. While garlic contained much less ascorbic acid, it had comparable effects on the radical scavenging.

Fig. 2. ESR spectra recorded 1 min after reaction initiation from a phosphate-buffered solution (pH 7.4) containing 100 mM DMPO and the following reactants: (a) 3.5 mM xanthine and 2 U/ml xanthine oxidase; (b) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 100 mg/ml apple juice; (c) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 100 mg/ml garlic juice; (d) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 100 mg/ml green pepper juice; (e) 3.5 mM xanthine, 2 U/ml xanthine oxidase, and 100 mg/ml orange juice. The ESR spectrometer settings were: receiver gain, $2.0 \times 10^4$; time constant 0.04 sec; modulation amplitude, 0.5 G; scan time, 60 sec; magnetic field, 3437 ± 100 G.
Antioxidant properties of fruit and vegetable juices

Table 1. Ability of natural fruit and vegetable juices to scavenge \*OH or O\(_2\)\* and to inhibit lipid peroxidation, and the correlation of these parameters with the ascorbic acid content of the fruit and vegetable juices

<table>
<thead>
<tr>
<th>Natural fruit or vegetable</th>
<th>Decrease in *OH (%)*</th>
<th>Decrease in O(_2)* (%)*</th>
<th>Decrease in lipid peroxidation (%)*</th>
<th>Ascorbic acid (mg/100 g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Apple</td>
<td>63.8 ± 0.2</td>
<td>75.9 ± 0.3</td>
<td>44.5 ± 2.3</td>
<td>5.1 ± 0.2</td>
</tr>
<tr>
<td>Blackberry</td>
<td>94.1 ± 0.3</td>
<td>90.7 ± 1.2</td>
<td>66.0 ± 3.1</td>
<td>18.3 ± 1.1</td>
</tr>
<tr>
<td>Broccoli</td>
<td>78.0 ± 0.4</td>
<td>83.3 ± 1.0</td>
<td>43.5 ± 4.5</td>
<td>113.5 ± 5.3</td>
</tr>
<tr>
<td>Cabbage</td>
<td>72.9 ± 0.1</td>
<td>95.3 ± 0.5</td>
<td>49.8 ± 2.7</td>
<td>46.8 ± 2.3</td>
</tr>
<tr>
<td>Carrot</td>
<td>58.7 ± 0.1</td>
<td>77.7 ± 0.4</td>
<td>34.4 ± 1.3</td>
<td>8.2 ± 0.5</td>
</tr>
<tr>
<td>Cucumber</td>
<td>68.4 ± 0.2</td>
<td>42.6 ± 0.7</td>
<td>34.7 ± 1.9</td>
<td>10.7 ± 1.2</td>
</tr>
<tr>
<td>Garlic</td>
<td>100.0 ± 0.3</td>
<td>94.4 ± 0.6</td>
<td>92.6 ± 3.2</td>
<td>15.3 ± 2.1</td>
</tr>
<tr>
<td>Grape, red</td>
<td>69.0 ± 0.5</td>
<td>76.8 ± 0.2</td>
<td>35.8 ± 1.4</td>
<td>4.5 ± 0.2</td>
</tr>
<tr>
<td>Grape, white</td>
<td>69.0 ± 0.2</td>
<td>65.7 ± 1.4</td>
<td>31.2 ± 2.1</td>
<td>3.8 ± 0.3</td>
</tr>
<tr>
<td>Green bean</td>
<td>94.8 ± 1.2</td>
<td>89.8 ± 2.2</td>
<td>76.1 ± 3.4</td>
<td>19.6 ± 1.9</td>
</tr>
<tr>
<td>Green pepper</td>
<td>94.2 ± 1.5</td>
<td>86.1 ± 1.6</td>
<td>79.9 ± 0.5</td>
<td>125.3 ± 5.1</td>
</tr>
<tr>
<td>Kiwi</td>
<td>90.9 ± 0.3</td>
<td>91.6 ± 0.4</td>
<td>70.3 ± 4.1</td>
<td>101.7 ± 3.7</td>
</tr>
<tr>
<td>Lemon</td>
<td>100.0 ± 0.9</td>
<td>96.3 ± 1.1</td>
<td>79.7 ± 2.9</td>
<td>49.2 ± 2.4</td>
</tr>
<tr>
<td>Lettuce, Romaine</td>
<td>95.5 ± 0.8</td>
<td>96.3 ± 0.9</td>
<td>81.0 ± 2.0</td>
<td>18.3 ± 0.9</td>
</tr>
<tr>
<td>Lime</td>
<td>100.0 ± 0.5</td>
<td>67.6 ± 0.7</td>
<td>81.7 ± 1.2</td>
<td>33.7 ± 1.9</td>
</tr>
<tr>
<td>Onion, white</td>
<td>93.5 ± 1.4</td>
<td>83.3 ± 0.4</td>
<td>59.9 ± 5.1</td>
<td>10.3 ± 1.3</td>
</tr>
<tr>
<td>Onion, yellow</td>
<td>88.4 ± 1.7</td>
<td>82.4 ± 1.0</td>
<td>56.5 ± 1.5</td>
<td>27.7 ± 2.6</td>
</tr>
<tr>
<td>Orange</td>
<td>98.0 ± 0.9</td>
<td>88.8 ± 1.2</td>
<td>77.1 ± 2.3</td>
<td>59.8 ± 4.2</td>
</tr>
<tr>
<td>Peach</td>
<td>69.0 ± 0.8</td>
<td>75.0 ± 0.6</td>
<td>35.0 ± 3.4</td>
<td>7.3 ± 0.5</td>
</tr>
<tr>
<td>Pear</td>
<td>39.3 ± 0.4</td>
<td>75.9 ± 0.7</td>
<td>33.6 ± 0.9</td>
<td>3.4 ± 0.2</td>
</tr>
<tr>
<td>Snow Pea</td>
<td>94.8 ± 0.3</td>
<td>83.3 ± 1.4</td>
<td>76.8 ± 1.4</td>
<td>10.6 ± 0.7</td>
</tr>
<tr>
<td>Spinach</td>
<td>81.9 ± 0.8</td>
<td>84.2 ± 2.0</td>
<td>55.4 ± 2.1</td>
<td>51.7 ± 6.2</td>
</tr>
<tr>
<td>Strawberry</td>
<td>100.0 ± 1.5</td>
<td>96.3 ± 0.3</td>
<td>77.5 ± 5.1</td>
<td>64.8 ± 3.7</td>
</tr>
<tr>
<td>Tomato</td>
<td>85.2 ± 1.1</td>
<td>61.1 ± 0.8</td>
<td>43.5 ± 3.0</td>
<td>22.4 ± 0.9</td>
</tr>
<tr>
<td>Watermelon</td>
<td>89.7 ± 0.9</td>
<td>68.5 ± 0.4</td>
<td>56.5 ± 1.8</td>
<td>9.5 ± 1.8</td>
</tr>
</tbody>
</table>

*All decreases were significant (p < 0.001)
Fig. 3 shows the mean (n = 3) values for ascorbic acid content, •OH peak height, O₂•⁻ peak height, and lipid peroxidation potency plotted against each other. The correlation (r²) for each curve was calculated. Curves plotting ascorbic acid content versus •OH radical peak height (Fig. 3A), O₂•⁻ radical peak height (Fig. 3B), and lipid peroxidation (Fig. 3C) demonstrated poor correlation values of 0.117, 0.145 and 0.118, respectively. These results indicate that ascorbic acid alone cannot explain the antioxidant activity of these natural food juices. The plot of •OH radical peak height vs magnitude of lipid peroxidation (Fig. 3D) demonstrated a good correlation (r² = 0.769), showing that the juices that were good •OH scavengers were also effective protectors against lipid peroxidation.

Discussion

ROS-mediated reactions have been found to be involved in the pathogenesis of a variety of diseases. Use of antioxidants is an important strategy against
diseases caused by free radical reactions. In recent years, there has been a growing interest in identifying potentially important antioxidants against free radicals, specifically those from naturally occurring substances. These substances include fruits and vegetables. Epidemiological studies have shown an association between the consumption of diets rich in fresh fruits and vegetables and decrease risks of cancer and other diseases [25]. While further studies need to be done, it is generally believed that the antioxidant constituents contribute to these protective effects. Our preliminary studies have indicated that certain polyphenolic compounds of the fruit and vegetable juices are responsible for the antioxidant activation. Further research is underway in our laboratory to identify the exact compounds.

It may be noted that ROS damage can be affected by two factors: (a) scavenging the radicals formed during reactions, and (b) inhibiting the reactions that form these radicals. Our investigation indicated that fruit and vegetable juices scavenged the radicals and did not inhibit their generation, as demonstrated by a spin trapping competition assay (data not shown).

H$_2$O$_2$, another important member of the ROS family, forms •OH from reactions with metal ions such as Fe(II). Chelation of such metals inhibits ROS damage by preventing these metals from reacting with H$_2$O$_2$ and forming •OH [26]. An organism can have many sources of free radicals, from production of O$_2$•− in mitochondria to production of H$_2$O$_2$ and •OH in macrophages [27]. Molecular oxygen (O$_2$) can generate O$_2$•− from the reaction involving NADPH and NADPH oxidase [28]. ROS damage occurs when there is an imbalance between the production of free radicals in the body and the body's antioxidant defenses [29]. Most juices tested in the present study have an acid pH; all experiments were carried out in PBS (pH 7.4) to buffer these reactions at a neutral pH.

Many health conscious individuals take antioxidant supplements to enhance the antioxidant capabilities of the body [30]. These antioxidants include vitamin C [31]. Our observations indicate that ascorbic acid is only one facet of the ROS scavenging capacity of fresh fruit and vegetable juices. Other free radical scavengers present in fruits and vegetables are flavonoids, carotenoids, organic acids (cinnamic acid and gallic acid), vitamin E, and sulfhydryl compounds [32]. These results suggest that eating fresh whole fruits and vegetables is a better way of obtaining antioxidants than taking a one-dimensional supplement. The present study shows that a variety of fruits and vegetables have antioxidant capabilities against •OH and O$_2$•−, the most common forms of ROS, and are potent inhibitors of the lipid peroxidation caused by these radicals. The present study also indicates that eating a well balanced diet of fruit and vegetables may enhance the antioxidant defenses against ROS-induced injuries to cells and tissues.

References


