CHANGES IN ANTIOXIDANTS DURING HEAT PROCESSING OF GREEN LEAFY VEGETABLES

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Received 27 August 2015; accepted 23 September 2015

Abstract
There is a growing interest in antioxidant rich foods for maintaining good health. Four green leafy vegetables namely, manathakkali, ponanganni, drumstick and mint were studied for retention of antioxidant compounds and their activity on cooking by boiling, pressure cooking, microwave cooking and stir frying similar to domestic cooking. This study indicates that mint is a very potent antioxidant food, followed by drumstick, ponanganni and manathakkali. Presence of poly phenols, flavonoids and tannins played a major role in their antioxidant activity. Stir frying of green leafy vegetables was found to be the best way to retain antioxidants. Significant losses in antioxidants occurred during dehydration of the leafy vegetables. This study implies that cooking and processing of foods should be such that valuable bioactive compounds are retained.

Key words: Green leafy vegetables; antioxidants; cooking; dehydration.

1. INTRODUCTION
With the awareness of the health benefits of dietary antioxidants, consumers prefer foods, particularly fruits and vegetables, with high antioxidant quality. This has also found new opportunities for the horticulture and food industry to improve the quality of their products by enhancing antioxidant content. Vegetables are normally consumed after being cooked. Therefore, it is important to know what happens to their antioxidant activity during common domestic processes (boiling, stir frying, pressure cooking, microwave cooking) and how much of it is really retained on cooking. This study discusses the effect of normal domestic cooking on the retention of antioxidants and their activities in four types of greens, namely manathakkali, ponanganni, drumstick and mint.

2. MATERIALS AND METHODS

2.1. Cooking methods
2.1.1. Boiling
Each green leafy vegetable (100g) was added to the 150 ml of distilled water in stainless steel vessel covered with a lid and cooked on a moderate flame at 100 °C for 10 min. It was drained off and cooled to room temperature.

2.1.2. Pressure cooking
Each green leafy vegetable (100g) was placed in stainless steel vessel containing 80 ml of distilled water and kept inside pressure cooker and cooked for 7 min. It was drained off and cooled to room temperature.

2.1.3. Microwave cooking
Each green leafy vegetable (100g) was placed in a plastic microwavable bowl with 60 ml of distilled water, covered with a lid and cooked in a microwave oven using high power for 5 min. It was cooled to room temperature.

2.1.4. Stir frying
Each green leafy vegetable (100g) was stir fried in a frying pan with 5 ml of hot refined sunflower oil for 2 min and 70 ml of distilled water was added and covered with a lid to prevent water loss and cooked on a low flame for 8 min. It was cooled to room temperature.

2.1.5. Dehydration
Green leafy vegetables (100g each) were steam blanched for 3 minutes and cooled to room temperature by dipping in cool water. Blanched leaves were spread on trays in single layer and dried in a cabinet dryer at 50±2°C to a moisture content of 5-6% in the finished product.
Table 1. Antioxidant components and antioxidant activity of fresh green leafy vegetables (Fresh weight basis)

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>Ascorbic acid (mg/100g)</th>
<th>Total Polyphenols (mg GAE/100g)</th>
<th>Total Flavonoids (mg CE/100g)</th>
<th>Tannin (mg TAE/100g)</th>
<th>Total Carotenoids (mg/100g)</th>
<th>DPPH radical scavenging activity (mg AEEA/100g)</th>
<th>Ferric Reducing Antioxidant Power (mg AEEAA/100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Manathakkali</td>
<td>14.1±0.32</td>
<td>128.50±2.8</td>
<td>87.32±2.62</td>
<td>65.20±0.88</td>
<td>3.81±0.01</td>
<td>204.41±2.54</td>
<td>102.71±4.57</td>
</tr>
<tr>
<td>leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Ponnanganni</td>
<td>24.20±0.13</td>
<td>233.89±0.08</td>
<td>115.07±2.85</td>
<td>35.00±1.57</td>
<td>5.85±0.03</td>
<td>48.00±1.41</td>
<td>38.82±1.32</td>
</tr>
<tr>
<td>leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drumstick</td>
<td>140.36±5.44</td>
<td>113.3±0.78</td>
<td>265.71±11.23</td>
<td>61.56±0.49</td>
<td>9.06±0.11</td>
<td>360.2±3.82</td>
<td>49.11±0.41</td>
</tr>
<tr>
<td>leaves</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mint leaves</td>
<td>30.20±0.41</td>
<td>365.26±12.84</td>
<td>317.44±0.11</td>
<td>83.53±3.04</td>
<td>4.37±0.02</td>
<td>323.60±3.82</td>
<td>174.40±2.43</td>
</tr>
<tr>
<td>S.ED</td>
<td>2.2338</td>
<td>5.3755</td>
<td>4.8521</td>
<td>1.4573</td>
<td>0.0503</td>
<td>2.0612</td>
<td>2.1910</td>
</tr>
<tr>
<td>CD (0.05)</td>
<td>5.1512**</td>
<td>12.3960**</td>
<td>11.1892**</td>
<td>3.3606**</td>
<td>0.1159**</td>
<td>4.7531**</td>
<td>5.0525**</td>
</tr>
</tbody>
</table>

2.2. Chemicals and reagents
All chemicals used were of analytical grade and purchased from Sisco Research Laboratories Pvt. Ltd., Mumbai, India.

2.3. Analysis of antioxidant components and activity
Ascorbic acid content was estimated by titration method and total polyphenols and tannins were determined by the spectrophotometric method, as described by Sadasivam and Manickam (2008). Total flavonoids were measured using aluminium chloride colorimetric assay, as described by Marinova et al. (2005). Total carotenoid content was determined as per the method described by Ranganna (1986). The analysis of antioxidant activity was carried out using DPPH assay, as per the method given by Goupy et al. (1999) and Ferric reducing antioxidant power (FRAP) assay by the modified method of Benzie and Strain (1996).

2.4. Statistical analysis
Data from all experiments were performed in triplicate for each sample. The results of the three replicates were pooled and expressed as mean ± standard deviation. Factorial completely randomized design (FCRD) as per method described by Gomez and Gomez (1984) was employed for analysis of data at 0.05 level of significance.

3. RESULTS
Table 1 indicates the antioxidant components and their antioxidant activities in fresh greens. Mint was the most potent antioxidant greens of the four types. It contained highest amounts of total polyphenols, total flavonoids and tannins. Manathakali scored the next in antioxidant activity, the least being ponnanganni.

3.1. Effect of cooking on antioxidant components of selected green leafy vegetables
Fig 1 shows that in the cooked greens very drastic losses in antioxidant activity were observed compared to the fresh samples. Drumstick greens contained distinctly high amounts of ascorbic acid amongst the four commonly consumed greens in all the treatments. On cooking, stir fried drumstick samples showed a small increase in ascorbic acid over the fresh sample. Similar increase in ascorbic acid on cooking was observed in the boiled and microwaved ponnanganni greens. Ascorbic acid is known for its solubility in water and it is easily lost in cooking medium and by oxidation. Pressure cooking and microwave cooking retained far less ascorbic acid in the greens. In Fig 2 stir fried greens indicate significantly higher values for total polyphenols. Pressure cooking and microwave cooking showed comparatively lower retention. Heating softens tissues thus releasing the phenols into solution. The total polyphenol content was the highest in mint in all the treatments. The increase was observed in all the samples, but microwave cooking showed the least retention in all the greens samples.

Total flavonoid content (Fig 3) was conspicuously high in all cooked samples of mint. Similar changes were found in all the greens but to a lesser extent. Stir frying brought about increase in total flavonoid content, while microwave cooking showed the least retention. It has been recognized that flavonoids show antioxidant activity and their effects on human nutrition and health are considerable. The mechanisms of action of flavonoids are through scavenging or chelating process (Kessler et al., 2003, Cook and Samman, 1996). All forms of cooking resulted in significantly higher amounts of tannins (Fig 4) compared to the uncooked greens, with microwave containing the least. Mint was found to contain strikingly high values of tannin in all the cooked samples, particularly stir frying. In fig 5 it is seen that total carotenoids were lost...
Table 2. Antioxidant activity of dehydrated green leafy vegetables (Dry weight basis)

<table>
<thead>
<tr>
<th>Vegetables</th>
<th>DPPH radical scavenging activity AAEAA (mg/ 100g)</th>
<th>Ferric Reducing Antioxidant Power AAEAA (mg/ 100g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fresh Dehydration</td>
<td>Fresh Dehydration</td>
</tr>
<tr>
<td>Manathakkali leaves</td>
<td>1135.61±1.63 25.18±0.09</td>
<td>570.61±21.60 24.37±0.13</td>
</tr>
<tr>
<td>Ponnanganni leaves</td>
<td>208.69±1.88 34.62±1.43</td>
<td>168.78±5.02 26.37±0.04</td>
</tr>
<tr>
<td>Drumstick leaves</td>
<td>222.20±9.01 48.15±0.82</td>
<td>204.62±3.68 43.52±0.90</td>
</tr>
<tr>
<td>Mint leaves</td>
<td>2157.33±12.78 104.42±4.23</td>
<td>1162.66±14.67 124.38±3.02</td>
</tr>
<tr>
<td></td>
<td>S.E.D CD (0.05)</td>
<td>S.E.D CD (0.05)</td>
</tr>
<tr>
<td>G</td>
<td>16.41236 34.79312**</td>
<td>5.51831 11.69845**</td>
</tr>
<tr>
<td>C</td>
<td>11.60529 24.60245**</td>
<td>3.90203 8.27205**</td>
</tr>
<tr>
<td>GC</td>
<td>23.21058 49.20490**</td>
<td>7.80407 16.54411**</td>
</tr>
</tbody>
</table>

Fig 3. Effect of cooking on total flavonoid content (mg CE/100g FWB) of selected green leafy vegetables

Fig 4. Effect of cooking on tannin content (mg TAE/100g FWB) of selected green leafy vegetables

on cooking by all the four methods in all the four greens samples. Only small differences were observed between the cooked samples, with drumstick showing better values of the four types of greens.

3.2. Effect of cooking on antioxidant activity of selected green leafy vegetables

Fig 6 shows the effect of cooking on the radical scavenging activity by DPPH assay of the selected greens. A significantly high antioxidant activity was seen in mint in all the treatments from fresh to all types of cooking. Here again there was a major increase in antioxidant activity on stir frying. The radical scavenging activity did not differ much in other greens in all the different treatments. FRAP assay of the greens in Fig 7 shows very similar changes as observed in DPPH assay; mint was found to exhibit far higher activity than the rest of the greens and stir frying indicating the highest value. All cooking treatments increased antioxidant activity in mint, the least being microwave cooking. Stir frying of the rest of the greens also showed better antioxidant activity than other cooking methods, with hardly any difference between the three greens.

3.3. Effect of dehydration on antioxidant components of selected green leafy vegetables

Effect of dehydration on the retention of antioxidant components is presented in fig 8. It shows that dehydration brings about appreciable losses in ascorbic acid, with the highest being in drumstick, though it had the highest value. Total polyphenols were lost on dehydration of greens and the greatest losses seen in mint. The total flavonoids showed maximum decrease in mint and manathakali on drying, though its content in mint was higher than that in the other greens. Marginal increase in flavonoids was noticed on dehydration of ponanganni and
Fig 5. Effect of cooking on total carotenoid content (mg/100g FWB) of selected green leafy vegetables

Fig 6. Effect of cooking on radical scavenging activity by DPPH assay (AAEAA mg/100 g FWB) of selected green leafy vegetables

Fig 7. Effect of cooking on radical scavenging activity by FRAP assay (AAEAA mg/100 g FWB) of selected green leafy vegetables

drumstick. Tannins content increased in drumstick, ponaganni and manathakali while it decreased in mint. Dehydration brought about an increase in carotenoids in drumstick and ponaganni and a decrease in manathakali and mint. Amongst the dehydrated samples mint was the best with two to four times the activity of that of the other samples.

3.4. Effect of dehydration on antioxidant activity of selected green leafy vegetables

Table 2 shows the effect of dehydration on the antioxidant activity of the greens. Significant reduction in antioxidant activity was noticed in all the greens to varying extents. This feature was found to be consistent even in FRAP assay. Of the four different greens mint possessed better antioxidant property. It was found that total flavonoids contributed most to the antioxidant property.

4. DISCUSSION

According to Sultana et al. (2007) the variations in antioxidant retention depend on the type of vegetable, cooking method, bioavailability of phenolics, temperature, location in the vegetables, cutting, stability of the structure to heat, the synergic activity of the structures, and on the antioxidant systems assayed (Yamaguchi et al., 2001). In boiling or pressure-cooking it is reported that lixiviation phenomenon occurs leading to 64% loss of total
carotenoids and 49% loss of total phenolics (Bunea et al., 2008). The phenols leach into the cooking water forming complex phenol proteins thus reducing drastically by 90% or more. The concentration of phenolic acids is highest in the outer layers of some vegetables (Turkmen et al., 2005) and these are extremely likely to be lost in cooking medium (Andlauer et al., 2003), reducing antioxidant power of some vegetables such as pea, spinach, cauliflower, and cabbage (Sultana et al., 2007). Total phenolics are usually stored in vegetables in pectin or cellulose networks and can be released during thermal processing. Contrary to our observation, microwave heating is reported to retain active components in the cooked tissue (Yamaguchi et al., 2001). The antioxidant activity of vegetables cooked in the microwave oven was reported to be generally higher than that of those cooked in boiling water, because microwave heating, griddling and baking does not stimulate the release of ascorbic acid or other antioxidants from cooked tissue. In the aqueous phase, oxygen, prooxidant metals, and water-soluble antioxidants, must diffuse through this phase. Therefore, differences in solubility and mobility and mass transfer rates of transition metals and antioxidants may significantly affect oxidation.

5. CONCLUSION

Our study recommends stir frying as the best method for cooking green leafy vegetables which are rich sources of not only nutrients but also antioxidant compounds that need to be retained on cooking. This information would help us to treat green leafy vegetables more gently to derive the health benefits from them.
ACKNOWLEDGEMENT

The authors gratefully acknowledge the financial support provided by University Grants Commission, New Delhi, India to conduct this research program.

REFERENCES


Source of support: Nil; Conflict of interest: None declared