Research article

Antioxidant properties of green broccoli and purple-sprouting broccoli under different cooking conditions

Yvette Porter*

School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, UK.
* Corresponding author: Email: yvetteporter@hotmail.co.uk
Supervisor: Prof. Khalid Rahman, School of Pharmacy and Biomolecular Sciences, Liverpool John Moores University, Liverpool, UK.

Diets rich in fruit and vegetables have long been associated with reduced risk of chronic disease. Antioxidant components of fruit and vegetables have recently generated great interest in scientific research. However, few studies have explored antioxidants in food after cooking. Cooking may alter antioxidant properties by initiating destruction, release or transformation of antioxidant food components. This study has investigated the effects of boiling and microwaving on the antioxidant properties of green broccoli and purple-sprouting broccoli. Antioxidant activities of the broccoli extracts were estimated using the 2,2-diphenyl-1-picrylhydrazyl radical scavenging method, vitamin C and phenols were estimated with the Folin–Ciocalteu reagent, flavonoids were evaluated using colorimetric methods and anthocyanins were determined by a pH differential method. Results showed antioxidant components of cooked broccoli to be quite different from uncooked broccoli. The antioxidant content of broccoli was retained and/or enhanced more after microwaving than after boiling. Cooking in water caused a leaching effect of antioxidants, and this increased with cooking time. Purple-sprouting broccoli was found to contain higher contents of antioxidant compounds compared with green broccoli, but tended to show higher sensitivity to cooking treatments. Cooking methods should be carefully considered in current dietary recommendations.

Key words: antioxidants, broccoli, vitamin C, polyphenols, flavonoids, anthocyanins

Submitted on 5 August 2011; accepted on 27 February 2012

Introduction

Diets rich in fruit and vegetables have long been associated with reduced risk of chronic disease, particularly cardiovascular disease, cancers and type 2 diabetes (Faller and Fialho, 2009). Oxidative stress from increased amounts of reactive oxygen species (ROS) can cause extensive damage to cell structures, and is considered a major factor in the pathogenesis of these chronic diseases (Roy et al., 2009). Evidence suggests that regular consumption of fruit and vegetables minimizes some of these harmful effects, which has been somewhat accredited to the presence of compounds possessing antioxidant properties (Podsedek, 2007). The major antioxidants present in fruit and vegetables are: vitamin C, vitamin E, carotenoids and polyphenols, especially flavonoids, which all provide protection against free radicals (Monero et al., 2010). The quality and quantity of these antioxidant components are major attributes to the health benefits of fruit and vegetables that are associated with reduced risk of chronic disease (Roy et al., 2009). For example, regular consumption of dark green leafy vegetables has shown a protective effect against two common eye diseases, cataract and macular degeneration, caused by free radicals generated by sunlight, metabolism and infection. These vegetables contain the pigments lutein and zeaxanthin, which accumulate in the eye and eradicate free radicals, thus preventing...
harm to the eye’s sensitive tissues (Christen et al., 2008). The high fibre content of fruit and vegetables also provide a protective effect. The bulking and softening action of indigestible fibre can reduce pressure inside the intestinal tract and calm the irritable bowel (Lembo and Camilleri, 2003).

Biological systems are constantly exposed to ROS generated both exogenously and endogenously. Accumulation of ROS can lead to damaging effects, through attacking DNA, proteins and lipids, which are central to the pathogenesis of chronic diseases (Valko et al., 2006). Deleterious effects of ROS are balanced by activities of antioxidants. Oxidative stress is the result of an imbalance of prooxidants (free radicals) and antioxidants in favour of prooxidants (Roy et al., 2009). Oxidative stress can be induced by variety of different factors, including UV radiation, metal-catalysed reactions, inflammation and electron transport reactions in the mitochondria (Valko et al., 2006). Antioxidant defences are imperative in biological protection against damage from ROS as they act as scavengers and directly remove free radicals (Pryor and Godber, 1991; Seis, 1997; Yamaguchi et al., 1998). Effective antioxidants can be enzymatic or non-enzymatic and exclusively quench free radicals, chelate radox metals and stimulate other antioxidants (Valko et al., 2006). Non-enzymatic antioxidants, including vitamin C, vitamin E, phenols and carotenoids, can be acquired through diet (Valko et al., 2006).

Broccoli belongs to the Brassica genus (Podsedek, 2007) and is renowned for its vast range of non-enzymatic bioactive compounds, being rich in both nutritional antioxidants; vitamins C and E, and non-nutritional antioxidants; carotenoids, and phenolic compounds, particularly flavanoids (Lin and Chang, 2005). Broccoli is also rich in polyphenols, a large group of phytochemicals that are often considered the most abundant antioxidants in the diet (Faller and Fialho, 2007). Polyphenols cause interference with oxidation of lipids and other molecules by the rapid donation of hydrogen atoms to free radicals. The intermediates of the phenoxy radical are fairly stable and so prevent the initiation of further radical reactions (Valko et al., 2006). Flavanoids and their derivatives are the largest and most prominent group of polyphenols and are ideal scavengers of peroxyl radicals due to their specific reduction actions on alkyl peroxyl radicals, making them effective inhibitors of liperoxidation (Valko et al., 2006). Broccoli has been reported to contain both flavonol and hydroxycinnamoyl derivatives (Vasanthi, Mukherjee and Das, 2009). Few studies have investigated anthocyanins in broccoli which are the most prominent group of plant pigments among the coloured flavonoids and possess high antioxidant activity (AA) (Monero et al., 2010). Monero et al. (2010) studied the properties of acylated anthocyanins in broccoli and found the colour of purple-sprouting broccoli to be the result of the presence of anthocyanins.

Another major health-promoting compound present in broccoli is vitamin C (Munyaka et al., 2010; Vasanthi, Mukherjee and Das, 2009). Vitamin C, which includes ascorbic acid and its oxidized product dehydroascorbic acid, participates in redox reactions in intra- and extracellular spaces of biological mechanisms. Vitamin C protects against cell death, directly scavenges superoxide radical, hydrogen peroxide, singlet oxygen and hydroxyl radicals, and acts as a lipid peroxidation chain-breaking agent (Gliszczynska-Swiglo et al., 2006). Vitamin C also co-operations with vitamin E to regenerate membrane-bound oxidized α-tocopherol, creating an ‘antioxidant network’ (Valko et al., 2006).

It is recognized that nutritional antioxidants act more efficiently in groups than singly, as they can perform as synergists to reduce ROS. Water-soluble antioxidants together with lipid-soluble antioxidants are able to quench free radicals in both their aqueous and lipid phases (Podsedek, 2007). Broccoli has been referred to as ‘The Crown Jewel of Nutrition’ since it possesses so many combinations of these health-promoting compounds, vitamins, minerals and fibre, therefore proclaiming its exceptional health benefits (Vasanthi, Mukherjee and Das, 2009).

The antioxidant content of broccoli varies significantly between and within its subspecies, which can be due to many factors, including genotype, growth conditions and storage conditions (Podsedek, 2007). In addition to this, domestic cooking can dramatically reduce activities of antioxidant components, as many of these compounds are very sensitive to heat and are soluble in water (Zhang and Hamauzu, 2004). Absorption of water during boiling can dilute and cause leaching of antioxidant compounds and thus decrease their antioxidant content (Podsedek, 2007).

There is a great amount of literature available concerning levels of antioxidant properties in raw fruit and vegetables (Sun et al., 2007; Gawlik-Dziki, 2008; Roy et al., 2009; Lemoine, Chaves and Martinez, 2010). However, there is less literature regarding the content of antioxidants in vegetables as usually eaten, i.e. after cooking. Variation in both cooking treatment and cooking duration may affect the nutritional value of vegetables (Lin and Chang, 2005). Broccoli is normally cooked by boiling in water or microwaving before consumption (Zhang and Hamauzu, 2004); thus, it is essential to determine which domestic cooking method and cooking duration are best for retaining antioxidants in this vegetable (Gliszczynska-Swiglo et al., 2006; Gebczynski and Lisiewsk, 2006).

The aim of this study was to investigate the effects of domestic cooking methods on the nutritional quality of both green broccoli and purple-sprouting broccoli by assessing the AA and content of vitamin C, phenols, flavanoids and anthocyanins of raw broccoli and then after boiling and microwaving, for different lengths of time.

**Materials and Methods**

**Chemicals and reagents**

The following chemicals and reagents were used: 2,2-diphenyl-1-picrylhydrazyl (DPPH), L-ascorbic acid, gallic acid, trichloroacetic acid, Folin–Ciocalteu reagent,
methanol (HPLC grade), sodium carbonate, catechin hydrate, sodium nitrite, sodium hydroxide, aluminium chloride, potassium chloride, sodium acetate, acetic acid and hydrochloric acid.

Plant materials
Fresh British green broccoli (Brassica oleracea) and purple-sprouting broccoli were obtained from a local Sainsbury’s supermarket. Florets and 6 cm of stems were used.

Cooking processes
Boiling
Three hundred millilitres of distilled water were heated to boiling. Broccoli (10 g) was added to the boiling water and cooked for 5, 10 and 20 min, before performing extractions.

Microwaving
Broccoli (10 g) was added to 100 ml of distilled water and cooked in a domestic microwave oven on a high heat for 1, 2 and 5 min, before performing extractions.

Extraction
Ten g (dry weight) of raw or cooked tissues were homogenized with 15 ml of 80% methanol. The homogenate was filtered through four layers of cheesecloth; the residue was homogenized with 15 ml of 80% methanol and then filtered again. This filtrate was treated and homogenized with a further 15 ml of 80% methanol and filtered for a third time. The filtrates were then centrifuged at 2000 g for 20 min. Supernatants were collected and diluted with distilled water to various concentrations. Extracts were stored in a refrigerator at 3°C for no longer than 4 weeks.

Antioxidant activity
Antioxidant activity was determined by the DPPH radical scavenging method of Zhang and Hamauzu (2004) with modifications. Essentially, 4 ml of 0.1 mM DPPH (in 80% methanol) solution was treated with 0.2 ml of extract, with the control containing 4 ml DPPH solution and 0.2 ml of distilled water, instead of the extract. The absorbance of the control containing 4 ml DPPH solution and 0.2 ml of distilled water, instead of the diluted reagent was added to the mixture and vigorously shaken. After 10 min at room temperature, the absorbance was measured at 760 nm against distilled water as a blank and the vitamin C content was estimated through the calibration curve of ascorbic acid.

Total phenols determination
The phenolic content of the obtained extracts was estimated by a colorimetric assay based on procedures by Singleton and Rossi (1965) with modifications. Forty per cent extract concentration (0.5 ml) was mixed with 0.5 ml of 0.2 M Folin–Ciocalteu’s phenol reagent. After 3 min, 0.5 ml of 7% aqueous sodium carbonate solution was added and the final volume was adjusted to 5 ml with distilled water. Mixtures were kept in darkness at room temperature for 90 min, and then absorbances were determined at 725 nm against distilled water as a blank. Results are expressed as the microgram of gallic acid equivalents/0.5 ml of extract (GAEs) through the calibration curve of gallic acid.

Flavonoid determination
The total flavonoid content of the obtained extracts was estimated using a colorimetric method described by Heimler et al. (2005), with modifications. Neat extracts of 0.25 ml were mixed with 75 µl of 5% sodium nitrite solution, 0.15 ml of freshly prepared 10% aluminium chloride solution and 0.5 ml of 1 M sodium hydroxide solution. The final volume of the mixture was adjusted to 2.5 ml with deionized water. The mixture was allowed to stand for 5 min at room temperature before the absorption was measured at 510 nm against the same mixture, minus the sample, as a blank. The total flavonoid content is expressed as (+)catechin equivalents [CE, µg (catechin/0.25 ml extract)] through the calibration curve of (+)catechin.

Anthocyanins determination
Anthocyanin content of the obtained neat extracts was determined using a pH differential method described by Hosseini, Li and Beta (2008). Two separate solutions of each samples were prepared, one for pH 1.0 using 0.03 M potassium chloride buffer, with hydrochloric acid (HCl) slowly added to the mixture to adjust the pH to 1.0. The other for pH 4.5 using 0.4 M sodium acetate buffer, using acetic acid to adjust the pH of the mixture to 4.5. The pH of the mixture was read using a calibrated pH meter. 0.5 ml of the sample was added to 1.5 ml of each buffer solution and
then adjusted to the suitable pH, as stated above. The absorbance of each mixture was measured at 520 nm against distilled water as a blank and the total anthocyanin content (µg/0.5 ml) was calculated using the following formula and expressed as Cy-3-glc equivalents:

\[
\frac{A \times MW \times DF \times 10^7}{\varepsilon \times L} = \text{Cy-3-glc equivalents (g/0.5 ml)}
\]

where \( A \) is the absorbance, \( (A\lambda \text{vis-max}) \) pH 1.0 – \( (A\lambda \text{vis-max}) \) pH 4.5, \( MW \) the molecular weight (g/mol) = 449.2 g/mol for Cy-3-glc, \( DF \) the dilution factor (0.4 ml of the sample is diluted to 2 ml DF = 5), and \( \varepsilon \) the extinction coefficient \( (L \times cm^{-1} \times mol^{-1}) = 26,900 \) for Cy-3-glc, where \( L \) (path length in cm) = 1.

**Statistical techniques**

Statistical analysis of the results was completed using Microsoft Office Excel 2007. Data are expressed as means ± standard deviation (SD) of a minimum of triplicate experiments taken from one extract of each separate cooking treatment. Differentiation between data sets was determined by Student’s \( t \)-test, and significant differences were considered when means of compared sets differed at \( P < 0.05 \).

**Results**

**Effects of cooking methods on AA**

Antioxidant activities of raw and cooked broccoli, as determined by the DPPH radical scavenging method, are shown in Table 1. Cooking method and duration affected the AA of both varieties of broccoli, with an overall more profound effect on purple-sprouting broccoli. Significant differences \( (P < 0.05) \) in AA were established between raw green and purple-sprouting broccoli after boiling and microwaving, but these differences became less significant with longer cooking times. Raw purple-sprouting broccoli showed dramatically higher AA (of 37.74%), compared with raw green broccoli, but cooking had a more unfavourable effect on the antioxidant content of purple-sprouting broccoli (Fig. 1). After boiling for 20 min and microwaving for 5 min, there was no significant difference in AA between the two varieties of broccoli (Table 1).

Initial microwaving for 1 min led to an increase in the AA of 6.46% in green broccoli; however, initial boiling for 5 min had no significant effect. AA in green broccoli either significantly decreased with cooking time or did not change significantly. Five minutes of boiling and 1 min of microwaving had a much greater effect on the AA of purple-sprouting broccoli, with losses of 51.65% and 17.72%, respectively. Boiling led to more unfavourable effects regarding AA, compared with microwaving in both varieties of broccoli.

The length of cooking times had a similar effect on the AA of green broccoli and purple-sprouting broccoli. Longer cooking time enhanced the reduction in AA of both varieties of broccoli (Fig. 1). Green broccoli retained 61.28% of raw total AA after 20 min of boiling and had significantly increased AA of 24.59% after 5 min of microwaving. Whereas purple-sprouting broccoli only retained 35.98% and 65.19% of total AA after boiling for 20 min and microwaving for 5 min, respectively. Initial cooking treatment had a greater effect on the AA of purple-sprouting broccoli, whereas the length of time cooked appeared to have greater effect on the AA of green broccoli.

**Effects of cooking methods on vitamin C content**

The content of vitamin C (ascorbic acid) dramatically decreased in both varieties of broccoli after cooking (Table 2). Raw green broccoli contained significantly more vitamin C than purple-sprouting broccoli, but cooking led to a greater loss of vitamin C in green broccoli. After cooking treatments, differences in the vitamin C content between green and purple-sprouting broccoli were either not significant or significantly higher in purple-sprouting broccoli, suggesting that the vitamin C content of green broccoli was more negatively affected by cooking.

**Table 1. Antioxidant activity of raw and cooked broccoli (% inhibition of DPPH)**

<table>
<thead>
<tr>
<th>Antioxidant activity (% inhibition)</th>
<th>Raw</th>
<th>Boiled</th>
<th>Microwaved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooking time (min)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green broccoli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>47.93 ± 4.61 a</td>
<td>58.45 ± 1.38 a</td>
<td>41.63 ± 1.88 a*</td>
</tr>
<tr>
<td>5</td>
<td>29.37 ± 0.80 a*</td>
<td>64.48 ± 3.43 a*</td>
<td>54.39 ± 3.23 a*</td>
</tr>
<tr>
<td>10</td>
<td>30.73 ± 2.18 a</td>
<td>70.27 ± 3.57 a*</td>
<td>61.35 ± 3.08 b*</td>
</tr>
<tr>
<td>20</td>
<td>3.43 ± 3.08 b*</td>
<td>55.72 ± 4.71 a*</td>
<td></td>
</tr>
<tr>
<td>Purple-sprouting broccoli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>85.40 ± 1.44 b</td>
<td>41.29 ± 1.19 b*</td>
<td>31.05 ± 2.34 b*</td>
</tr>
<tr>
<td>5</td>
<td>30.73 ± 2.18 a</td>
<td>70.27 ± 3.57 a*</td>
<td>61.35 ± 3.08 b*</td>
</tr>
<tr>
<td>10</td>
<td>3.08 b*</td>
<td>55.67 ± 1.04 a*</td>
<td></td>
</tr>
</tbody>
</table>

Data are means of triplicate experiments taken from one extract of each cooking treatment ± SD.

*Significant differences between lengths of cooking times, within cooking methods (boiled 5 min and microwaved 1 min extracts are compared with raw extracts).

Different letters in the same column indicate significant differences between varieties of broccoli exposed to the same cooking treatment \( P < 0.05 \).
It is clear from Fig. 2 that boiling had an overall greater effect on vitamin C loss than microwaving. Boiling for 5 min led to a loss of 61.94% and 1 min of microwaving led to a loss of 52.65% of vitamin C in green broccoli. In purple-sprouting broccoli, a loss of 52.8% and 18.88% was detected after boiling for 5 min and microwaving for 1 min, respectively.

The vitamin C content of green broccoli decreased continually with cooking time for both cooking methods. Whereas the vitamin C content of purple-sprouting broccoli significantly increased from boiling for 5–10 min, but then significantly decreased after 20 min. When the purple-sprouting broccoli was microwaved, the vitamin C content decreased from cooking times 1 to 2 min and then did not significantly change from 2 to 5 min.

### Effects of cooking methods on total phenolic, flavonoid and anthocyanin content

The effects of boiling on total phenolic content and flavonoid content of green and purple-sprouting broccoli are presented in Fig. 3, and the effect of microwaving is presented in Fig. 4. Both cooking methods affected the phenolic content and the flavonoid content of broccoli. Total phenolics and flavonoids of raw broccoli were both dramatically higher in purple-sprouting broccoli, compared with green broccoli (24.38 µg/0.5 ml extract and 17.41 µg/0.25 ml extract higher, respectively); however, both cooking methods had a more deleterious effect on the total phenolic and flavonoid content of purple-sprouting broccoli compared with green broccoli.
Boiling for 5 min led to a 59.77% reduction in total phenols and a 49.55% reduction in flavonoids in purple-sprouting broccoli. Total phenols and flavonoids decreased further again after boiling for 10 min, and then did not significantly change between 10 and 20 min of boiling time (Fig. 3). Microwaving had a similar effect on purple-sprouting broccoli, but lead to greater losses of flavonoids compared with phenols (Fig. 4).

Boiling significantly reduced the total phenolic concentration of green broccoli with cooking time, resulting in a 27.68%, 46.79% and 60.41% loss of phenols after 5, 10 and 20 min of boiling, correspondingly. However, boiling had no significant effect on the flavonoid content of green broccoli (Fig. 3). Microwaving for 2 min caused a significant decrease of 37.5% phenols in green broccoli, but microwaving for 1 and 5 min had no significant effect. Microwaving had no...
significant effect on the flavonoid content of green broccoli (Fig. 4).

Associations between the total phenolic and flavonoid content of broccoli after cooking treatment, particularly in the results for purple-sprouting broccoli, can be seen in Figs 3 and 4. Changes in the total phenolic and flavonoid content appear to follow similar patterns when exposed to different cooking treatments.

The total phenolic content and the total anthocyanin content, determined by the pH differential method, in raw green and raw purple-sprouting broccoli were compared and the results are shown in Fig. 5. Both total phenolic and anthocyanin content were found to be dramatically higher in raw purple-sprouting broccoli compared with raw green broccoli. The anthocyanin content of raw purple-sprouting broccoli was found to be 6-fold more than that of green broccoli, and the total phenolic content was found to be nearly double that of green broccoli.

**Discussion**

**AA of broccoli under different cooking conditions**

Broccoli is widely considered to possess high levels of AA (Roy et al., 2009); however, specific values for AA of broccoli vary greatly between studies. Fresh broccoli used in this study was found to possess AA of 47.93% (green) and 85.40% (purple sprouting), by reduction in DPPH free radical. Turkman, Sari and Velioglu (2005) found fresh broccoli to show AA of 78.17% and Zhang and Hamauzu (2004) recorded an average of 60.5%. These disparities could be explained by differences in the concentration of bioactive compounds between varieties of broccoli (Vasanthi, Mukherjee and Das, 2009), differences between florets and...
stems within varieties and may also have been influenced by differences in harvesting and environmental conditions.

This study found AA of green broccoli to significantly increase with initial exposure to cooking, but then decrease with cooking time. Turkman, Sari and Velioğlu (2005) and Wachtel-Galor, Wong and Benzie (2008) also found AA to increase during cooking, whereas Zhang and Hamauzu (2004) reported a decline in AA during cooking. Gliszczynska et al. (2006) found the antioxidant activities of broccoli to decrease when cooked in water, but increase when steamed. These observations support the suggestion that an increase in AA after cooking may be a result of enhanced availability for extraction by more efficient release of antioxidants compounds from intracellular proteins, altered cell wall structures and matrix modifications (Wachtel-Galor, Wong and Benzie, 2008), and a reduction in AA is expected to be due to antioxidant compounds leaching into the cooking water (Gliszczynska et al., 2006). Despite the dramatically higher AA of raw purple-sprouting broccoli, its instability with heat does not compensate for this, as AA declined continuously with cooking. This is likely to be due to diversity in antioxidant compounds between the varieties, which can vary in thermal stability (Watchel-Galor, Wong and Benzie, 2008) and affinity to the DPPH radical.

For both varieties of broccoli, the AA was significantly lower when cooked by boiling than when cooked by microwaving. However, Watchel-Galor, Wong and Benzie (2008) reported AA of four Brassica vegetables (including broccoli) to be lower after microwaving than boiling. This study (Watchel-Galor, Wong and Benzie, 2008) used a different method for the estimation of AA, by the ferric reducing/antioxidant power assay and also used different cooking times, which may explain the contrasting results. Three studies (Zhang and Hamauzu, 2004; Turkman, Sari and Velioğlu, 2005; Faller and Fialho, 2009) found no significant differences in the total AA of broccoli, after boiling and microwave cooking. These studies also applied shorter cooking times compared with the present study, which may help explain the lack of differences.

It is important to acknowledge, when comparing data, that effects of cooking on AA can depend on many additional factors such as variations in cooking procedures, temperatures of heating, solvents used for extraction, pH of reactions and exposure to water and oxygen (Watchel-Galor, Wong and Benzie, 2008). In addition, Zhang and Hamauzu reported differences in vitamin C between the florets and stems of broccoli, which may have influenced dissimilarities in reported values. Research has confirmed that concentrations of biologically active compounds, including ascorbic acid and phenols, largely contribute to AA of vegetables (Borowski et al., 2008).

**Vitamin C content of broccoli under different cooking conditions**

Cooking treatments led to deleterious effects on the vitamin C content of both varieties of broccoli. Similar effects were observed by Zhang and Hamauzu (2004) and Gliszczynska-Swigło et al. (2006), both reporting dramatic losses in the vitamin C content in broccoli after boiling and microwave cooking. However, the loss of vitamin C after 5 min of boiling reported in the present study is >2-fold observed by Gliszczynska-Swigło et al. (2006). According to available data, vitamin C levels can vary over 4-fold, between and within subspecies of broccoli (Podsedek, 2007) which may help explain variability between data.

Differences in analytical methods can also result in varied data. The Folin reagent method used in this study focuses on ascorbic acid as a marker of vitamin C; however, other forms of vitamin C, such as dehydroascorbic acid (DHA), are not accounted for. DHA is a product of oxidized ascorbic acid which other studies have found to contribute to the vitamin C content of vegetables (Podsedek, 2007). Vallejo, Tomás-Barberan and García-Viguera et al. (2003) found DHA to contribute 11.3% to the total vitamin C content of broccoli; however, ascorbic acid was still reported to be the dominant form of vitamin C.

Boiling had a greater effect on vitamin C loss compared with microwaving. Observations by Yuan et al. (2009) were similar, and also reported steaming to have no significant effect on the vitamin C content, as did Vallejo, Tomás-Barberan and García-Viguera et al. (2002). Vitamin C is highly water-soluble, so cooking in water may cause greater losses by leaching into surrounding water than by methods such as steaming (Yuan et al., 2009). Overall results from this study and others indicate that cooking influences retention of vitamin C in the cells of broccoli, due to its high solubility in water and low stability with heat (Francisco et al., 2010).

**Total phenolic, flavonoid and anthocyanin content of broccoli under different cooking conditions**

Results from the present study found both cooking methods to exhibit a negative effect on the phenolic content of broccoli, with boiling causing a more profound effect. Other studies (Watchel-Galor, Wong and Benzie, 2008; Francisco et al., 2010) also reported the depletion of phenolics in Brassica vegetables after boiling and microwaving, but Watchel-Galor, Wong and Benzie (2008) found steaming to retain the phenolic content of broccoli, cauliflower, cabbage and choy sum. Phenolic compounds in vegetables are present in both soluble forms and combined with cell wall complexes. Thus, increased surface area of tissues in contact with cooking water, as well as high cooking temperatures and lengthy cooking times are all likely to have caused disruption of cell walls and breakdown of phenolic compounds (Francisco et al., 2010). Results concerning steamed vegetables in the study by Watchel-Galor, Wong and Benzie (2008) may have been influenced by the lower temperatures of steaming which may not have affected the phenolic content as greatly. Zhang and Hamauzu (2004) reported much greater phenolic losses of 71.9% and 71.6% in broccoli after 5 min of cooking by boiling and microwaving. However, Turkman, Sari and
Velioglu (2005) reported no detrimental effect of total phenolic content in various green vegetables after boiling and reported the total phenolic content in broccoli to increase after steaming and microwaving. Differences in extraction and cooking procedures can contribute towards the array of contrasting results, proving comparisons between studies to be very difficult.

The phenolic content of purple-sprouting broccoli was considerably higher than green broccoli but was more negatively affected by both cooking treatments. Different varieties of broccoli contain different phenolic compounds, with specific bonds and arrangements within cells. Therefore, cleavage of phenolic bonds differs between varieties, according to the degree of heat applied (Faller and Fialho, 2009).

Results showed changes in flavonoid content during cooking to follow similar patterns as phenolic content, suggesting that flavonoids contribute to the phenolic content of broccoli. The present study found both cooking methods to dramatically reduce the flavonoid content of purple-sprouting broccoli, with boiling having more pronounced effects. Other studies (Gawlik-Dziki, 2008; Faller and Fialho, 2009; Francisco et al., 2010) also reported a diminishing effect of flavonoids in vegetables after cooking. Whereas results regarding the flavonoid content of green broccoli show no significant difference between raw and cooked extracts. Although the green broccoli retained more flavonoids after cooking, the flavonoid content of purple-sprouting broccoli was still significantly higher. The much higher levels of anthocyanins observed in the purple-sprouting broccoli are likely to have largely contributed to the high flavonoid content. This observation was expected as anthocyanins are renowned for their red/purple pigmentation (Monero et al., 2010), and evidently give rise to the colour of the purple-sprouting broccoli. Other studies, including Turkman, Sari and Velioglu (2005), have presented divergent results, reporting heat treatment to cause an increase in the concentration of free flavonoids in vegetables. Flavonoids show different behaviours depending on the cooking procedure performed (Faller and Fialho, 2009), which is likely to have contributed to the ambiguous results between and within studies.

Limitations

The DPPH method used in this study to estimate AA is based on the ability of antioxidants to reduce the DPPH free radical. Although the method allows simple and rapid measurement of free-radical scavenging activity, it has the limitation of colour interference and sample solubility (Oboh et al., 2010). Therefore, AA of broccoli needs further investigation using a more versatile radical, for example, ABTS.

In regard to the colorimetric assay for total phenols determination, some reducing components, including ascorbic acid, can be oxidized by the Folin–Ciocalteu reagent and seen as phenolics (Zhang and Hamauzu, 2004). Interference of compounds may have affected the validity of results, and thus further assays are required to establish the phenolic content of broccoli. High-performance liquid chromatography could also have been applied for the isolation and identification of individual phenolic compounds.

This study investigated effects of two common cooking methods (boiling and microwaving) on antioxidant properties. Other methods including steaming were not assessed due to time constraints. Evidence from other studies found steaming to retain and often increase antioxidant properties (Gawlik-Dziki, 2008; Roy et al., 2009). Effects of steaming on the antioxidant properties of vegetables, along with other cooking methods, need to be further investigated to establish definite effects. Compounds present in the cooking water could also have been investigated to verify leaching actions.

The activities of food antioxidant components have been assessed in vitro. In vitro measurements cannot mimic biological conditions and do not account for metabolic reactions, which largely manipulates the bioavailability of antioxidant components. Clearly, much work is needed to establish antioxidant effects in vivo to confirm beneficial activities of these compounds. Plasma antioxidant capacity has been suggested as a good in vivo marker of antioxidant status (Fernandez-Patchon et al., 2008).

Conclusion

The results presented in this study evidently showed that cooking causes dramatic alterations in the antioxidant properties of broccoli. This is expected to have resulted from an array of effects, including damage, release and transformation of food components. In this study, microwaving generally retained more antioxidant components of both varieties of broccoli, compared with boiling. This could be explained by longer cooking times, larger volumes of water and potentially higher temperatures during boiling. Cooking in water seems to cause a leaching effect of antioxidants, and this increases with cooking time. Using cooking water for other uses, for example, soups and gravies, as well as alternative cooking methods, such as steaming, should be considered for the optimal intake of antioxidants from cooked vegetables (Podsedek, 2007).

Overall, purple-sprouting broccoli was found to contain higher contents of antioxidant compounds than green broccoli, but tended to show higher sensitivity to cooking treatments. After cooking, purple-sprouting broccoli retained higher antioxidant status and therefore may have a more beneficial effect, but at a much higher retail price. Further bioavailability studies are warranted to ascertain these data.

The general decrease in antioxidant properties observed in this study indicates that the actual intake of health-promoting compounds is overestimated when using data from raw vegetables; therefore, cooking methods should be considered in current dietary recommendations. Further research is needed to increase knowledge regarding the bioavailability of antioxidant compounds from vegetables, and to confirm effects of different domestic cooking methods on the concentration and structure of these compounds.
Acknowledgements

Great thanks to my Project Supervisor, Khalid Rahman, for all of his help and support throughout my project. Thanks to Peter Hamlett, Senior Technician, for his assistance during experimental work in the laboratory.

Author biography

Y.P. studied BSc Nutrition at Liverpool John Moores University and graduated in July 2011 with a First-Class Degree with Honours. Y.P. also won the Yakult award for best Nutrition student and is currently seeking a career in Nutrition. Y.P. has a particular interest in Nutaceuticals and aspires to work in this field in the future.

References


Munyaka, A. W., Oey, I., Loey, A. V. et al. (2010) Application of thermal inactivation of enzymes during vitamin C analysis to study the influence of acidification, crushing and blanching on vitamin C stability in broccoli (Brassica oleracea L var. Italica), Food Chemistry, 120 (2), 591–598.


florets after domestic cooking, European Food Research and Technology, 215 (4), 310–316.


