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Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking

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Abstract

Antioxidant components, including phenolics, ascorbic acid and carotenoids, of broccoli floret and stem, antioxidant activity, and their changes during conventional and microwave cooking, were investigated. Broccoli florets and stem were cooked by conventional boiling or by microwave over up to 300 s. Total phenolics were retained by up to 28.1–28.4% in the cooked florets and 55.6–57.8% in the cooked stems, and ascorbic acid by 34.1–34.4% and 29.1–29.5%, respectively. Total carotenoids were retained better compared to total phenolics and ascorbic acid. Total antioxidant activity was retained at 34.7–35.0% in the cooked florets and 34.6–34.7% in the cooked stems and phenolic antioxidant activity was retained at 37.4% and 64.7%, respectively. The results showed that antioxidant components and antioxidant activity in broccoli were lost heavily during the cooking. These losses need to be taken into account when calculating the dietary intake of these compounds from the cooked broccoli.

Keywords: Phenolics; Ascorbic acid; Carotenoids; Antioxidant activity; Conventional and microwave cooking

1. Introduction

Fruits and vegetables are good sources of natural antioxidants for the human diet, containing many different antioxidant components which provide protection against harmful free radicals and have been strongly associated with reduced risk of chronic diseases, such as cardiovascular disease, cancer, diabetes, Alzheimer's disease, cataracts and age-related functional decline in addition to other health benefits (Cao, Sofic, & Prior, 1996; Cohen, Kristal, & Stanford, 2000; Knekt et al., 2002; Liu et al., 2000; Sweeney, Kalt, Mackinnon, Ashby, & Gottschall-Pass, 2002; Velioglu, Mazza, Gao, & Oomah, 1998; Wang, Cao, & Prior, 1996). These antioxidants include carotenoids, vitamins, flavonoids, other phenolic compounds, dietary glutathione, and endogenous metabolites (Larson, 1988).

Many studies have shown that a frequent intake of cruciferous vegetables, such as broccoli, cauliflower, leaf mustard, cabbage, Chinese broccoli, and turnip, could be helpful in protecting against cancer (De Long, Prochaska, & Talalay, 1986; Prochaska, Santanmaria, & Talalay, 1992; Zhang, Taylor, Kramer, & Li, 1995). Broccoli is becoming increasingly popular as a fresh vegetable and is a significant source of nutritional antioxidants, such as vitamins and carotenoids, as well as biologically active dietary components, such as the flavonol glycosides (Hertog, Hollman, & Katan, 1992; Plumb, Price, Rhodes, & Williamson, 1997; Price, Casuscelli, Colquhoun, & Rhodes, 1998), hydroxycinnamic acids (Price, Casuscelli, Colquhoun, & Rhodes, 1997) and sulphur-containing compounds, such as the glucosinolates (Rose, Heaney, Fenwick, & Portas, 1997).

It is common that many vegetables are cooked by a simple boiling process or microwave process before use. These cooking processes would certainly bring about a number of changes in physical characteristics and chemical composition of vegetables (Sukhwant,

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Harvinder, & Tejinder, 1992). Broccoli is normally cooked by a boiling or microwave process before being eaten. Khachik et al. (1992) reported that various cooking procedures affected the carotenoid content of in green vegetables. Price et al. (1998) pointed out that cooking affected the phenolic content of broccoli. However, the information on antioxidant components, antioxidant activity and their changes during cooking is still limited. Therefore, that the effect of cooking on qualitative and quantitative distribution of antioxidant components should be investigated.

The objective of this study was to investigate phenolics, ascorbic acid and carotenoids, antioxidant activity of broccoli floret and stem and their changes during conventional and microwave cooking.

2. Materials and methods

2.1. Plant materials

Broccoli (*Brassica olearacea*) bunches were obtained from a local wholesale market in Minami-minowa, Nagano. The bunch was separated into florets and stems. The floret and stem were chopped into small pieces with a knife before cooking or extraction.

2.2. Cooking processes

2.2.1. Conventional cooking

200 ml of water in a 400 ml beaker were heated to boiling. The beaker was covered with a plate, preventing water loss. 10 g of broccoli floret or stem were added to the boiling water and cooked for 30, 60, 90, 120, and 300 s. The samples were drained off before the succeeding extractions.

2.2.2. Microwave cooking

10 g of broccoli floret or stem were added to 200 ml of boiled water (over 80 °C at the beginning) in a 400 ml beaker and then cooked in a domestic microwave oven (Panasonic 600 W power) for 30, 60, 90, 120, and 300 s. The beaker was also covered with a plate, preventing water loss. The samples were drained off before the succeeding extractions.

2.3. Determination of phenolics

10 g of fresh or cooked tissues were homogenized with 15 ml of 80% methanol. The homogenate was filtered through four layers of cheesecloth and the residue was treated with 15 ml of 80% methanol for two successive extractions. The filtrates were combined and centrifuged at 4000*g* for 10 min. Methanol was removed from the supernatant by evaporation under vacuum at 35 °C, pigments and fatty acids were eliminated by two successive extractions with petroleum ether (2:1, v:v). The aqueous phase was collected, acidified to pH 2.0 and then loaded into a Sep-Pak C18 cartridge preconditioned with methanol and acidic water. After washing with distilled water, the cartridge was eluted with methanol. The methanol solution was concentrated under vacuum at 35 °C for determination of phenolics and antioxidant property. Phenolics were determined by the Folin–Ciocalteu reagent, as described by Singleton and Rossi (1965), using gallic acid as a standard and expressed as milligramme gallic acid equivalents (GAE)/100 g fresh weight (FW).

2.4. Ascorbic acid assay

10 g of fresh or cooked tissues were homogenized with 15 ml of 5% metaphosphoric acid. The homogenate was filtered with four layers of cheesecloth and the residue was treated with 10 ml of 5% metaphosphoric acid for two successive extractions. The filtrate was combined and centrifuged at 4000*g* for 10 min. The supernatant was collected and made up to 50 ml and then filtered through a 0.45 μm Advantec filter for HPLC analysis. HPLC analysis was performed using a SHIMADZU LC-10A Liquid Chromatograph with a Shodex Asahipak NH2P-50 4E column (250 × 4.60 mm from Showa Denko K.K. Japan) at 40 °C. The mobile phase consisted of acidified distilled water (0.1% phosphoric acid) (solvent A) and acetonitrile (solvent B), at a ratio of 25:75. The flow rate was 1.0 ml/min. L-ascorbic acid was detected at 254 nm.

2.5. Carotenoid analysis

Carotenoids were analyzed according to the methods of Howard, Talcott, Brenes, and Villalon (2000), Mizda, Shinmoto, Kobori, and Tsushida (2002) with some modification. 15 g of fresh or cooked tissues were homogenized with 10 ml of −20 °C acetone. The homogenate was filtered with four layers of cheesecloth and the residue was treated with −20 °C acetone for three successive extractions until the green colour could no longer be visually detected in the extract and residue. The filtrate was combined and centrifuged at 4000*g* for 10 min. The supernatant was collected and then filtered through a 0.45 μm Advantec filter for HPLC analysis. Samples were separated on a Luna 5μ C18 column (150 × 4.60 mm from Phenomenex) at 40 °C by an HPLC (SHIMADZU LC-VP Liquid Chromatograph equipped with a SPD-M10Avp photodiode array detector). The mobile phase consisted of acetonitrile-water (9:1, solvent A) and ethyl acetate (solvent B). The flow rate was 1.0 ml/min. The following gradient elution was used for separation: 0% B at 0 min, 50% B at 20 min, and 50% B at 35 min. Samples were detected at 450 nm. Identification of carotenoids was tentatively achieved by

their retention times and UV spectra, recorded with a SHIMADZU SPD-M10Avp photodiode array detector, in comparison with authentic β-carotene and carotenoids, identified according to Howard et al. (2000) and Mizda et al. (2002). The content of carotenoids was expressed as milligramme β-carotene equivalents per 100 g FW.

2.6. Antioxidant activity

10 g of fresh or cooked tissues were homogenized with 15 ml of 80% methanol. The homogenate was filtered through four layers of cheesecloth and the residue was treated added with 15 ml of 80% methanol for two successive extractions. The filtrates were combined and centrifuged at 4000*g* for 10 min. The supernatant of the methanol extract was collected and diluted to various concentrations (1.0%, 2.5%, 5%, 7.5%, and 10%) for measurement of total antioxidant activity. After the samples at various concentrations were studied, the concentration representing 5% of original fresh tissue weight was chosen as an appropriate concentration for assessing antioxidant activity in all of the fresh or cooked samples. Antioxidant activity was determined by the 1,1-diphenyl-2-picryl-hydrazyl (DPPH) method of Brand-Williams, Cuvelier, and Berset (1995) with some modification. An 0.1 mM solution of DPPH in methanol was prepared and 4 ml of this solution were treated with 0.2 ml of the diluted extract. A control was treated with 0.2 ml of distilled water instead of the extract. The mixture was left to stand at room temperature for 60 min before the decrease in absorbance at 517 nm was measured. Antioxidant activity was expressed as the percentage of DPPH decrease using the equation:

$$\text{AA } (\%) = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \times 100 \quad (1)$$

Phenolic antioxidant activity was also measured at the concentration representing 5% of original fresh plant tissue weight by the same method so as to assess the phenolic contribution to total antioxidant activity.

3. Results

3.1. Effect of cooking on total phenolics, ascorbic acid, and carotenoids in broccoli floret and stem

Fresh broccoli floret contained 7.7 times more phenolics than the stem (Table 1). The effect of cooking on total phenolics in broccoli floret and stem are shown in Table 1. The florets cooked conventionally for 30, 60, 90, 120, and 300 s lost 31.6%, 47.5%, 55.9%, 61.7%, and 71.9% of total phenolics present in the fresh floret, respectively, while the cooked stems lost 13.3%, 22.2%, 26.7%, 28.9%, and 42.2%, respectively. In the microwave cooking, the change of total phenolics in both florets and stems showed a similar trend.

Fresh broccoli stem contained of 124 mg/100 g FW ascorbic acid which was 19.8% higher than the floret (Table 2). The cooking caused loss of ascorbic acid in broccoli floret and stem (Table 2). The florets cooked conventionally for 30, 60, 90, 120, and 300 s lost 19.2%, 33.5%, 47.5%, 59.2%, and 65.9% of ascorbic acid present in the fresh florets, respectively, while the cooked stems lost 19.1%, 33.4%, 47.7%, 59.2%, and 70.9%, respectively. In the microwave cooking (for 300 s), the florets and stems lost 65.6% and 70.5%, respectively, of ascorbic acid.

Broccoli floret mainly contained lutein, β-carotene, and violaxanthin, representing 54.7% of total carotenoids. The stem contained 0.10 mg/100 g FW of carotenoids, less than 3% of total carotenoids present in the floret (Table 3). The effect of cooking on carotenoids in broccoli floret and stem is shown in Table 3. Both conventional and microwave cooking caused loss of total carotenoids in broccoli florets and stems. The florets cooked conventionally for 30, 60, 90, 120, and 300 s lost 2.7%, 12.0%, 14.4%, 17.1%, and 22.9% of total carotenoids, respectively, while the stems cooked for 60, 120, and 300 s lost 10.0%, 20.0% and 20.0%,

Table 1
Effect of cooking on total phenolics in broccoli floret and stem[a]

Cooking process and broccoli parts	Total phenolics (mg/100 g FW)					
	Cooking time (s)					
	0	30	60	90	120	300
Conventional						
Floret	34.5 ± 1.0	23.6 ± 0.7	18.1 ± 0.6	15.2 ± 0.5	13.2 ± 0.5	9.7 ± 0.4
Stem	4.5 ± 0.2	3.9 ± 0.2	3.5 ± 0.2	3.3 ± 0.1	3.2 ± 0.1	2.6 ± 0.1
Microwave						
Floret	34.5 ± 1.0	24.1 ± 0.7	18.5 ± 0.7	15.5 ± 0.6	13.2 ± 0.5	9.8 ± 0.4
Stem	4.5 ± 0.2	4.0 ± 0.2	3.7 ± 0.1	3.3 ± 0.1	3.1 ± 0.1	2.5 ± 0.1

[a] Data are means of three duplicate experiments ±SD.

Table 2
Effect of cooking on ascorbic acid in broccoli floret and stem[a]

Cooking process and broccoli parts	Ascorbic acid (mg/100 g FW)					
	Cooking time (s)					
	0	30	60	90	120	300
Conventional						
Floret	103 ± 3.5	83.5 ± 2.6	68.7 ± 2.1	54.2 ± 2.0	42.1 ± 1.9	35.2 ± 1.5
Stem	124 ± 3.8	100 ± 3.5	82.5 ± 2.7	64.7 ± 2.1	50.5 ± 1.9	36.0 ± 1.5
Microwave						
Floret	103 ± 3.5	85.1 ± 2.5	70.2 ± 2.1	54.3 ± 2.1	43.1 ± 1.8	35.5 ± 1.4
Stem	124 ± 3.8	103 ± 3.5	85.0 ± 2.5	65.2 ± 2.1	50.9 ± 1.8	36.5 ± 1.5

[a] Data are means of three duplicate experiments ±SD.

Table 3
Effect of cooking on carotenoids in broccoli floret and stem[a]

Cooking process	Carotenoids	Cooking time (s)					
		0	30	60	90	120	300
Conventional							
Floret	Total	3.75 ± 0.25	3.65 ± 0.25	3.30 ± 0.23	3.21 ± 0.23	3.11 ± 0.20	2.89 ± 0.20
	Lutein	1.05 ± 0.15	1.08 ± 0.16	1.13 ± 0.15	1.18 ± 0.16	1.23 ± 0.15	1.33 ± 0.17
	β-Carotene	0.63 ± 0.13	0.35 ± 0.05	0.15 ± 0.04	0.13 ± 0.04	0.13 ± 0.04	0.13 ± 0.04
	Violaxanthin	0.37 ± 0.06	0.31 ± 0.06	0.28 ± 0.05	0.26 ± 0.05	0.24 ± 0.04	0.17 ± 0.03
Stem	Total	0.10 ± 0.03	0.10 ± 0.03	0.09 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02
Microwave							
Floret	Total	3.75 ± 0.25	3.70 ± 0.25	3.35 ± 0.24	3.23 ± 0.23	3.10 ± 0.20	2.90 ± 0.20
	Lutein	1.05 ± 0.15	1.07 ± 0.15	1.14 ± 0.15	1.18 ± 0.16	1.22 ± 0.16	1.33 ± 0.17
	β-Carotene	0.63 ± 0.13	0.38 ± 0.06	0.15 ± 0.04	0.14 ± 0.04	0.13 ± 0.04	0.13 ± 0.04
	Violaxanthin	0.37 ± 0.06	0.33 ± 0.05	0.30 ± 0.05	0.27 ± 0.05	0.24 ± 0.04	0.18 ± 0.03
Stem	Total	0.10 ± 0.03	0.10 ± 0.03	0.10 ± 0.03	0.08 ± 0.02	0.08 ± 0.02	0.08 ± 0.02

[a] Amounts are expressed in mg/100 g FW. Data are means of three duplicate experiments ±SD.

respectively. In the microwave cooking, total carotenoids in the florets and stems also declined continuously. The florets cooked in microwave for 120 and 300 s lost 17.3% and 22.7% of total carotenoids, respectively, while the cooked stem lost 20.0%. The level of β-carotene in the florets declined during the conventional and microwave cooking, 76.2% of β-carotene was lost in the first 60 s. On the other hand, the level of lutein increased gradually during the conventional and microwave cooking. The level increased by 26.7% during the cooking for 300 s.

3.2. Effect of cooking on antioxidant activity in broccoli floret and stem

Total antioxidant activity and antioxidant activity of phenolic extracts was measured by the DPPH method. Broccoli floret and stem extracts were found to possess

Table 4
Effect of cooking on antioxidant activity in broccoli floret and stem [a]

Cooking process		Antioxidant activity (%)					
		0 s	30 s	60 s	90 s	120 s	300 s
Conventional							
Floret	Total	60.5 ± 2.5	48.9 ± 2.2	40.2 ± 2.0	32.8 ± 1.8	23.7 ± 1.3	21.2 ± 1.3
	Phenolics	12.1 ± 0.8	8.5 ± 0.5	7.0 ± 0.5	6.1 ± 0.6	5.4 ± 0.5	4.5 ± 0.5
Stem	Total	62.8 ± 2.5	50.8 ± 2.3	41.8 ± 2.1	32.8 ± 1.8	24.7 ± 1.4	21.8 ± 1.3
	Phenolics	1.7 ± 0.2	1.5 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.2 ± 0.1	1.1 ± 0.1
Microwave							
Floret	Total	60.5 ± 2.5	50.1 ± 2.2	41.5 ± 2.1	32.8 ± 1.9	23.8 ± 1.3	21.0 ± 1.3
	Phenolics	12.1 ± 0.8	8.7 ± 0.6	7.1 ± 0.5	6.1 ± 0.5	5.5 ± 0.5	4.5 ± 0.5
Stem	Total	62.8 ± 2.5	53.0 ± 2.3	42.6 ± 2.0	33.1 ±	24.8 ± 1.3	21.7 ± 1.3
	Phenolics	1.7 ± 0.2	1.6 ± 0.2	1.3 ± 0.2	1.2 ± 0.2	1.2 ± 0.1	1.1 ± 0.1

[a] The extracts for antioxidant activity were tested at a level representing 5% of original fresh plant material weight. Data are means of three duplicate experiments ±SD.

good antioxidant activity. Phenolic extract accounted for 20.0% and 2.7% of total antioxidant activity in the florets and stems, respectively (Table 4). The effect of cooking on antioxidant activity in broccoli floret and stem is shown in Table 4. Total antioxidant activity and phenolic antioxidant activity of broccoli florets and stems declined during the conventional and microwave cooking. During the conventional cooking, the florets retained 80.8%, 66.4%, 54.2%, 39.2%, and 35.0% of total antioxidant activity after being cooked for 30, 60, 90, 120, and 300 s, respectively, while the stems retained 80.9%, 66.6%, 52.2%, 39.3% and 34.7%, respectively. Antioxidant activities of the phenolic extracts were retained at 70.2%, 57.9%, 50.4%, 44.6%, and 37.2% in the florets, respectively, while the stems retained 88.2%, 76.5%, 70.6%, 70.6%, and 64.7%, respectively. In the microwave cooking, the changes of antioxidant activity showed a similar trend. The florets and stems, in microwave cooking for 300 s, retained 34.7% and 34.6% of total antioxidant activity and 37.2% and 64.7% of phenolic antioxidant activity, respectively.

4. Discussion

Data on total phenolics in broccoli are very limited. Leja, Mareczek, Starzynska, and Rozek (2001) reported that broccoli florets contained 56.2 mg/100 g FW of total phenolics. In the present study, broccoli florets were found to contain 34.5 mg/100 g FW less than that reported by Leja et al. (2001). The difference could be explained by removal of interfering components, such as ascorbic acid, from the extract using a reverse phase Sep-Pak C18 column in the present study. Some reducing components, such as ascorbic acid, capable of being oxidized by the Folin–Ciocalteu reagents of the total phenolic assay, yield the reduced (coloured) forms of the reagents and appear as phenolics (Waterman & Mole, 1994). The different varieties used might also cause differences. In addition, differences in ascorbic acid and carotenoids, between florets and stems, were also observed in the present study. The results indicate that phenolics, ascorbic acid, and carotenoids are distributed differently in broccoli floret and stem.

Ewald, Fjelkner-Modig, Johnsson, Sjoholm, and Akesson (1999) reported that, for blanched onions, green beans and peas, various cooking procedures including boiling, microwaving, frying or further warmholding, did not significantly affect the levels of quercetin and kaempferol. However, Price et al. (1998) reported that, after cooking for 15 min, only 18% of phenolic compounds in broccoli were retained in the cooking tissue, the remainder being largely leached into the cooking water. The present study found that both broccoli florets and stems turned soft and were ready for eating after cooking in boiling water for 5 min. Total phenolics declined continuously during the cooking. The florets cooked for 5 min by boiling or microwave cooking retained 28.1% and 28.4% of total phenolics, respectively. The results indicated that cooking caused loss of phenolics and phenolics were largely leached into the cooking water.

Khachik et al. (1992) reported that, in the steaming and microwave cooking for 5 min, the content of total carotenoids remained unchanged, while the level of lutein and β-carotene increased and violaxanthin declined. However, our results showed that total carotenoids in broccoli florets and stems declined during cooking. β-Carotene in the florets was quite heat labile and a substantial level of this compound was lost in the first 60 s of the cooking. On the other hand, the level of lutein increased by 26.7% during the cooking for 5 min. This could be due to transformation of the *cis* isomer of lutein to the *trans* form (Britton & Hornero-Mendez, 1997; Khachik et al., 1992). Some workers have reported losses of β-carotene from vegetables, including spinach, amaranth and fenugreek, during cooking procedures, such as boiling, stewing, frying, blanching and pressure cooking (Yadav & Sehgal, 1995, 1997).

Fennema (1997) pointed out that the cooking procedures could result in significant losses of vitamin C. Yadav and Sehgal (1995, 1997) reported losses of ascorbic acid from vegetables including spinach and fenugreek, during cooking procedures, such as boiling, stewing, frying, blanching and pressure cooking. The results obtained in the present study showed that the content of ascorbic acid declined dramatically during both conventional and microwave cooking. This indicates that cooking affects retention of ascorbic acid in the tissues.

It has been reported that phenolic compounds, ascorbic acid and carotenoids can contribute to antioxidant activity (Byers & Perry, 1992; Frei, England, & Ames, 1989; Velioglu et al., 1998). Free radical-scavenging is one of the known mechanisms by which antioxidants inhibit lipid oxidation caused by free radicals. Racchi et al. (2002) reported that boiling significantly reduced antioxidant activity in mushroom juice, affected that in yellow bell pepper and onion juices slightly, but did not affect that in white cabbage juice. In the present study, total antioxidant activity declined, along with antioxidant components, during the cooking. The phenolic antioxidant activity also showed similar trends. These findings indicate that the cooking could also result in significant losses of antioxidant activity due to antioxidants being largely leached into the cooking water.

Zia-ur-Rehman, Islam, and Shah (2003) reported that conventional and microwave cooking made no significant difference to the contents of fibre, cellulose, hemicellulose and lignin of 10 vegetables. Khachik et al. (1992) reported that no significant difference in total

carotenoids between steaming and microwave cooking, would be observed. Ewald et al. (1999) reported that there were no significant differences in the contents of quercetin and kaempferol between boiling and microwave cooking. The results obtained in the present study showed no significant differences in the contents of antioxidant components and antioxidant activity between conventional and microwave cooking.

Lu and Foo (2000) reported that flavonoid glycoside conjugates were better than epicatechin, phloridzin, chlorogenic acid, ascorbic acid and vitamin E as DPPH free radical scavengers, and flavonoid aglycones, such as quercetin, were higher in radical-scavenging activity than their glycosides. Broccoli contained flavonol glycosides and hydroxycinnamic acids (Hertog et al., 1992; Plumb et al., 1997; Price et al., 1997, 1998). Though antioxidant activity in phenolic extracts only accounted for 20.0% and 2.7% of total antioxidant activity in florets and stems, respectively, flavonoid glycoside conjugates would be degraded to aglycones by human intestinal flora (Leighton et al., 1992) and could provide higher radical-scavenging activity and antioxidant activity.

In conclusion, antioxidant components, such as phenolics, ascorbic acid and carotenoids, in broccoli and antioxidant activity have been shown to be heavily lost during cooking. These losses need to be taken into account when calculating the dietary intake of these compounds from cooked broccoli. In addition, the cooking conditions used here are probably extreme, and are likely to be experienced. In other processes, such as blanching and stir frying, the extents of loss would be lower.

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References

Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free radical method to evaluate antioxidant activity. *Lebensmittel-Wissenschaft und Technologie, 28*, 25–30.

Britton, G., & Hornero-Mendez, D. (1997). Carotenoids and colour in fruit and vegetables. In: F. A. Tomas-Barberan, & R. J. Robins (Eds.), *Phytochemistry of fruit and vegetables. Proceedings of the phytochemical society of Europe* (pp. 11–27). Oxford: Clarendon Press.

Byers, T., & Perry, G. (1992). Dietary carotenes, vitamin C, and vitamin E as protective antioxidants in human cancers. *Annual Review of Nutrition, 12*, 139–159.

Cao, G., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry, 44*, 3426–3431.

Cohen, J. H., Kristal, A. R., & Stanford, J. L. (2000). Fruit and vegetable intakes and prostate cancer risk. *Journal of National Cancer Institute, 92*, 61–68.

De Long, M. J., Prochaska, H. J., & Talalay, P. (1986). Induction of NAD(P)H: Quinine reductase in murine hepatoma cells by phenolic antioxidants, azo dyes, and other chemoprotectors: A model system for the study of anticarcinogens. *Proceedings of the National Academy of Science of the United States of America, 83*, 787–791.

Ewald, C., Fjelkner-Modig, S., Johnsson, K., Sjoholm, I., & Akesson, B. (1999). Effect of processing on major flavonoids in processed onions, green beans, and peas. *Food Chemistry, 64*, 231–235.

Fennema, O. (1997). Loss of vitamins in fresh and frozen foods. *Food Technology, 31*(12), 32–38.

Frei, B., England, L., & Ames, B. N. (1989). Ascorbate is an outstanding antioxidant in human blood serum. *Proceedings of the National Academy of Science of the United States of America, 86*, 6377–6381.

Hertog, M. G. L., Hollman, P. C. H., & Katan, M. B. (1992). Content of potentially anticarcinogenic flavonoids of 28 vegetables and 9 fruits commonly consumed in the Netherlands. *Journal of Agricultural and Food Chemistry, 40*, 2379–2383.

Howard, L. R., Talcott, S. T., Brenes, C. H., & Villalon, B. (2000). Changes in phytochemical and antioxidant activity of selected pepper cultivars (*Capsicum* species) as influenced by maturity. *Journal of Agricultural and Food Chemistry, 48*, 1713–1720.

Khachik, F., Goli, M. B., Beecher, G. R., Holden, J., Lusby, W. R., Tenorio, M. D., & Barrera, M. (1992). Effect of food preparation on qualitative and quantitative distribution of major carotenoid constituents of tomatoes and several green vegetables. *Journal of Agricultural and Food Chemistry, 40*, 390–398.

Knekt, P., Kumpulainen, J., Jarvinen, R., Rissanen, H., Heliovaara, M., Reunanen, A., Halulinen, T., & Aromaa, A. (2002). Flavonoids intake and risk of chronic diseases. *American Journal of Clinical Nutrition, 76*, 560–568.

Larson, R. A. (1988). The antioxidants of higher plants. *Phytochemistry, 4*, 969–978.

Leighton, T., Ginther, C., Fluss, L., Harter, W. K., Cansado, J., & Notorio, V. (1992). Molecular characterization of quercetin and quercetin glucosides in Allium vegetables. In M. T. Huang, C. T. Ho, & C. Y. Lee (Eds.), *Phenolic compounds in food and their effects on health II* (pp. 220–238). Washington, DC: American Chemical Society.

Leja, M., Mareczek, A., Starzynska, A., & Rozek, S. (2001). Antioxidant ability of broccoli flower buds during short-term storage. *Food Chemistry, 72*, 219–222.

Liu, S., Manson, J. E., Lee, I. M., Cole, S. R., Hennekens, C. H., Willett, W. C., & Burning, J. (2000). Fruit and vegetable intake and risk of cardiovascular disease: The Women's Health Study. *American Journal of Clinical Nutrition, 72*, 922–928.

Lu, Y., & Foo, Y. (2000). Antioxidant and radical scavenging activities of polyphenols from apple pomace. *Food Chemistry, 68*, 81–85.

Mizda, Y., Shinmoto, H., Kobori, M., & Tsushida, T. (2002). Sepaeration and quantification of carotenoids and chlorophylls in vegetables by high-performance liquid chromatography. *Journal of Japanese Society for Food Science and Technology, 49*(7), 500–506.

Plumb, G. W., Price, K. R., Rhodes, M. J., & Williamson, G. (1997). Antioxidant properties of the major polyphenolic compounds in broccoli. *Free Radical Research, 27*(4), 429–435.

Price, K. R., Casuscelli, F., Colquhoun, I. J., & Rhodes, J. C. (1997). Hydroxycinnamic acid esters from broccoli florets. *Phytochemistry, 45*, 1683–1687.

Price, K. R., Casuscelli, F., Colquhoun, I. J., & Rhodes, J. C. (1998). Composition and content of flavonol glycosides in broccoli florets (*Brassica olearacea*) and their fate during cooking. *Journal of the Science of Food and Agriculture, 77*, 468–472.

Prochaska, H. J., Santanmaria, A. B., & Talalay, P. (1992). Rapid detection of inducers of enzymes that protect against carcinogens. *Proceedings of the National Academy of Science of the United States of America, 89*, 2394–2398.

Racchi, M., Daglia, M., Lanni, C., Papetti, A., Govoni, S., & Gazzani, G. (2002). Antiradical activity of water soluble components in common diet vegetables. *Journal of Agriculture and Food Chemistry, 50*, 1272–1277.

Rose, E. A. S., Heaney, R. K., Fenwick, G. R., & Portas, C. A. M. (1997). Glucosinolates in crop plants. *Horticultural Reviews, 19*, 99–216.

Singleton, V. L., & Rossi, J. A., Jr (1965). Colorimetry of total phenolics with phosphomolybdic–phosphotugstic acid reagents. *American Journal of Enology and Viticulture, 16*, 144–158.

Sukhwant, M. K., Harvinder, K., & Tejinder, G. (1992). Effect of cooking on fibre content of vegetables. *Journal of Food Science and Technology, 29*, 185–186.

Sweeney, M. I., Kalt, W., Mackinnon, S. L., Ashby, J., & Gottschall-Pass, K. T. (2002). Feeding rats diets enriched in lowbush blueberries for six weeks decreases ischemia-induced brain damage. *Nutritional Neuroscience, 5*(6), 427–431.

Velioglu, Y. S., Mazza, G., Gao, L., & Oomah, B. D. (1998). Antioxidant activity and total phenolics in selected fruits, vegetables, and grain products. *Journal of Agricultural and Food Chemistry, 46*, 4113–4117.

Wang, H., Cao, G., & Prior, R. L. (1996). Total antioxidant capacity of fruits. *Journal of Agricultural and Food Chemistry, 44*, 701–705.

Waterman, P.G. & Mole, S. (1994). Extraction and chemical quantification. In: Waterman, P.G., & Mole, S. (Ed.), *Analysis of phenolic plant metabolites*, (pp. 66–103).

Yadav, S. K., & Sehgal, A. (1995). Effect of home processing on ascorbic acid and beta-carotene content of spinach(*Spinachia oleracia*) and amaranth (*Amaranthus tricolor*) leaves. *Plant Foods for Human Nutrition, 47*, 125–131.

Yadav, S. K., & Sehgal, A. (1997). Effect of home processing on ascorbic acid and beta-carotene content of bathua(*Chenopodium album*) and fenugreek (*Trigonella foenumgraecum*) leaves. *Plant Foods for Human Nutrition, 50*, 239–247.

Zhang, Y. H., Taylor, P. R., Kramer, T. R., & Li, J. Y. (1995). Possible immunologic involvement of antioxidants in cancer prevention. *American Journal of Clinical Nutrition, 62*, 1477–1482.

Zia-ur-Rehman, Islam, M., & Shah, W. H. (2003). Effect of microwave and conventional cooking on insoluble dietary fibre components of vegetables. *Food Chemistry, 80*, 237–240.