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Antioxidant activity and phenolic content of raw and blanched *Amaranthus* species

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Abstract

The study was aimed to determine the antioxidant activity (total antioxidant and free radical-scavenging activities) and total phenolic content of *Amaranthus* sp. The effects of different blanching times (10 and 15 min) on antioxidant activity and phenolic content were also studied. Four types of *Amaranthus* species locally known as spinach, namely 'bayam putih' (*Amaranthus paniculatus*) (BP), 'bayam merah' (*Amaranthus gangeticus*) (BM), 'bayam itik' (*Amaranthus blitum*) (BI) and 'bayam panjang' (*Amaranthus viridis*) (BPG), were selected. Total antioxidant activity of water-soluble components in raw spinach was in the order of BI ≈ BM ≈ BPG > BP, whereas free radical-scavenging activity was in the order of BI > BPG > BM > BP. The total phenolic contents of BM and BP were significantly higher ($p < 0.05$) than other samples. All the studied spinach species possessed different antioxidant activities and phenolic contents. Antioxidant activities and phenolic contents of all the spinach were in the order of raw > blanched 10 min > blanched 15 min. Blanching up to 15 min may affect losses of antioxidant activity and phenolic content, depending on the species of spinach.

Keywords: Spinach; Blanching; Total antioxidant activity; Scavenging activity; Phenolic content

1. Introduction

Overwhelming scientific data, from epidemiological studies, indicate that diets rich in fruit, vegetables and grains are associated with a lower risk of several degenerative diseases, such as cancers (Steinmetz & Potter, 1996) and cardiovascular diseases (Rimm et al., 1996). This association is often attributed to different antioxidant components, such as vitamin C, vitamin E, carotenoids, lycopenes, polyphenols and other phytochemicals.

Food composition data, necessary for epidemiological and nutritional studies, are merely representative of foodstuffs consumed in the raw state. Many food composition databases never take into consideration the fact that concentrations of nutrients and their activity may change through cooking practices such as blanching. This is of great importance, considering that only a small amount of vegetables is consumed in the raw state, whilst most need to be processed for safety and quality.

Amaranthus sp., locally known as spinach or "bayam", is one of the most popular leafy vegetables consumed in Malaysia. Five types of spinach species can be found in Malaysia; 'bayam putih' (*Amaranthus paniculatus*), 'bayam merah' (*Amaranthus gangeticus*), 'bayam itik' (*Amaranthus blitum*), 'bayam duri' (*Amaranthus spinosus*), and 'bayam panjang' (*Amaranthus viridis*). However, only four species of spinach, namely 'bayam putih', 'bayam merah', 'bayam itik' and 'bayam panjang', are abundantly available in the market, and commonly consumed by urban or rural Malaysians. The vegetable has been reported to have a high concentration of antioxidant

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components (Hunter & Fletcher, 2002). This leafy vegetable is generally cooked before being consumed. Losses of antioxidant components from vegetables during cooking have been reported elsewhere (Chu, Chang, & Hsu, 2000; Yadav & Sehgal, 1995). However, no research has been carried out to investigate the antioxidant properties of these four species of spinach. This study was first initiated to determine the antioxidant activity and polyphenol content of these vegetables. However, our previous study found that the total antioxidant activity and phenolic content in 'bayam putih' significantly decreased after 1 min of blanching (Amin, Zamaliah, & Chin, 2004). In practice, spinach is cooked with water for quite some time before being consumed. Hence, the effects of blanching times (10 and 15 min) on the loss of antioxidant activity and polyphenol content were also estimated.

2. Materials and methods

2.1. Samples

2.1.1. Material

Four types of healthy and fresh spinach species, namely 'bayam putih' (*Amaranthus paniculatus*), 'bayam merah' (*Amaranthus gangeticus*), 'bayam itik' (*Amaranthus blitum*) and 'bayam panjang' (*Amaranthus viridis*), were randomly selected and purchased from several retailers in the wholesale market at Seri Kembangan, Selangor, Malaysia.

2.1.2. Preparation of sample

Spinach was cleaned under running tap water and excessive water was drained off. The spinach (1 kg) was chopped into small pieces and divided into three portions (raw, blanching for 10 min, and blanching for 15 min). Blanching was done by simmering the vegetables in boiling water in the ratio of 1 to 5, draining the sample and leaving it to cool at room temperature. All the raw and blanched samples were lyophilized using a bench top freeze-dryer (Labconco, Freezone 4.5, USA). The lyophilized sample was homogenised using a blender (National; MX-291N, Kuala Lumpur, Malaysia) before being transferred into an air-tight container, and kept at −20 °C for further analysis.

2.1.3. Preparation of extract

The ground sample was extracted with distilled water in the ratio of 1 to 10. The mixture was placed in a conical flask (wrapped with an aluminium foil) and agitated at 100 rpm with the aid of an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h. The mixture was then filtered through a Whatman No. 4 filter paper to obtain a clear extract. The water extract was used for all analysis.

2.2. Measurement of antioxidant activity

2.2.1. Total antioxidant activity

β-Carotene bleaching assay was carried out according to the method developed by Wettasinghe and Shahidi (1999). Briefly, 2 ml β-carotene solution (0.2 mg/ml chloroform) were pipetted into a round-bottom flask containing 20 μl linoleic acid and 200 μl Tween 20. The mixture was then evaporated at 40 °C for 10 min using a rotary evaporator (Laborata 4000, Heidolph Instruments GmbH & Co. KG, Germany) to remove chloroform. After evaporation, the mixture was immediately added to 100 ml of distilled water. The mixture was vigorously agitated to form an emulsion.

Five millilitre aliquots of the emulsion were transferred into different test tubes containing 200 μl of extract. The mixture was then gently mixed and placed in a water bath at 50 °C for 2 h. Absorbance of the sample was measured every 15 min for 2 h at 470 nm using a Spectronic® Genesys™ 5 spectrophotometer (Milton Roy Company, New York, USA). Blank solution was prepared, containing the same concentration of sample without β-carotene. All determinations were performed in triplicate. The total antioxidant activity was calculated based on the following equation:

$$\mathrm{AA} = \left[1 - \frac{A_0 - A_t}{A_0^{\circ} - A_{\mathrm{t}}^{\circ}}\right] \times 100,$$

where AA is antioxidant activity, A_0 and A_0° are the absorbance values measured at initial time of the incubation for samples and control, respectively, while A_{t} and A_t° are the absorbance values measured in the samples or standards and control at $t = 120$ min.

2.2.2. Free radical-scavenging activity

Effect of extract on DPPH free radical was measured, based on Lee, Park, and Choi (1996). Positive control was prepared by mixing 4 ml of ascorbic acid (0.05 mg/ml) and 1 ml of DPPH (0.4 mg/ml), whereas negative control was prepared by mixing distilled water with 1 ml of DPPH.

Four millilitre of the extract (a final concentration of 20 mg/ml) were added to 1 ml DPPH. The mixture was gently homogenized and left to stand at room temperature for 30 min. Absorbance was read using a spectrophotometer at 520 nm. The ability of extract to scavenge DPPH free radical was calculated using the following equation.

$$\text{Scavenging activity}(\%) = \left[\frac{A(-\mathrm{ve}) - A_{\mathrm{s}}}{A(-\mathrm{ve}) - A(+\mathrm{ve})}\right] \times 100,$$

where, As is the absorbance of the sample, $A(-\mathrm{ve})$ and $A(+\mathrm{ve})$ are the absorbance values of negative and positive controls, respectively.

2.2.3. Measurement of total phenolic content

Total polyphenol content was estimated using Folin–Ciocalteu assay, developed by Velioglu, Mazza, Gao, and Oomah (1998), with slight modifications. Briefly, 0.75 ml of Folin–Ciocalteu reagent (1:9; Folin–Ciocalteu reagent:distilled water) and 100 μl of sample (5 mg/ml) were put into a test tube. The mixture was mixed and allowed to stand for 5 min at room temperature. Then, 0.75 ml sodium carbonate solution was added, followed by 10 ml of distilled water. The mixture was homogenized and allowed to stand at room temperature for 90 min. Total polyphenol content was determined using a spectrophotometer at 725 nm. Gallic acid was used as the standard, and total polyphenol content was expressed as gallic acid equivalents (GAE) g/kg of fresh spinach.

2.3. Statistical analysis

Data were expressed as means ± standard deviation of three measurements. One-way ANOVA was used to analyse the mean differences between raw and blanched spinach species. A significant different was considered at the level of $p < 0.05$.

3. Results

3.1. Total antioxidant activity

Figs. 1–3 show the β-carotene bleaching rates of the four raw and blanched spinach extracts. Trolox, used as the standard, had a higher antioxidant activity than spinach extracts or control. A decrease in absorbance, for all the samples compared with the standard, indicates that all the studied spinach species possessed lower antioxidant capacity. The absorbance values for all the samples decreased with incubation time.

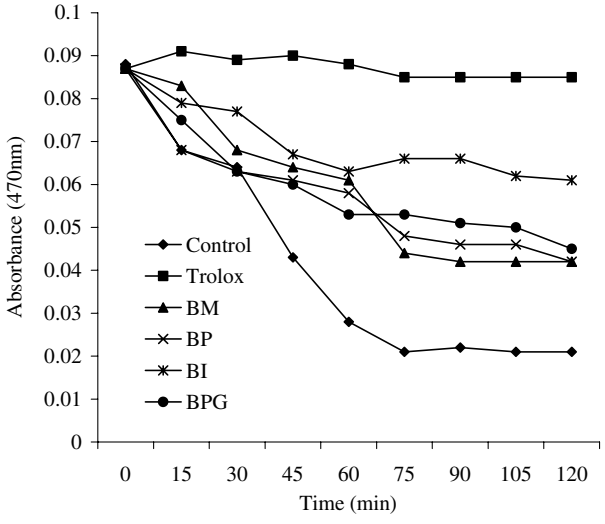

Fig. 1. Antioxidant activity of raw spinach extracts using a β-carotene–linoleate model system: BM, bayam merah; BP, bayam putih; BI, bayam itik; BPG, bayam panjang.

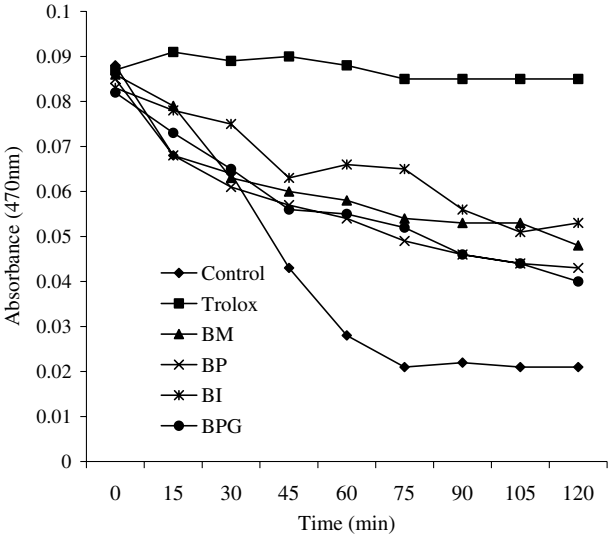

Fig. 2. Antioxidant activity of 10 min blanched spinach extracts using a β-carotene–linoleate model system: BM, bayam merah; BP, bayam putih; BI, bayam itik; BPG, bayam panjang.

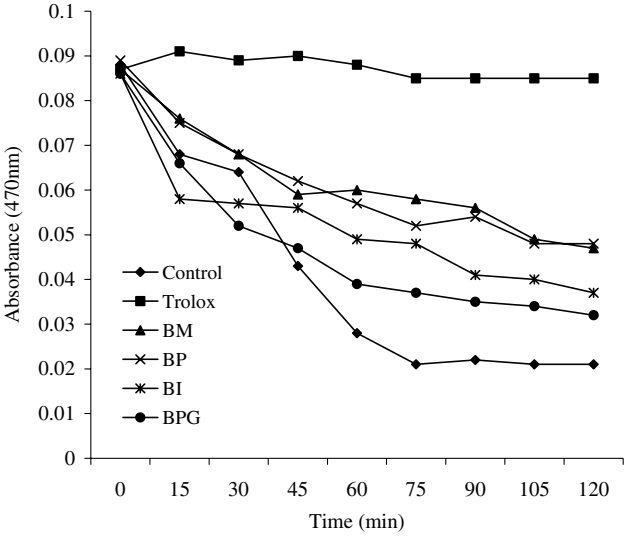

Fig. 3. Antioxidant activity of 15 min blanched spinach extracts using a β-carotene–linoleate model system: BM, bayam merah; BP, bayam putih; BI, bayam itik; BPG, bayam panjang.

Fig. 4 shows the mean total antioxidant activity of raw and blanched spinach species. The means of total antioxidant activity for raw 'bayam itik' (BI), 'bayam merah' (BM), 'bayam panjang' (BPG) and 'bayam putih' (BP) were 61 ± 4%, 59 ± 1%, 52 ± 9% and 44 ± 3%, respectively. The results showed that BI had the highest total antioxidant activity, followed by BM, BPG and BP.

As shown in Fig. 4, the total antioxidant activities of BI, BM, BPG and BP decreased to 56 ± 1%, 44 ± 1%,

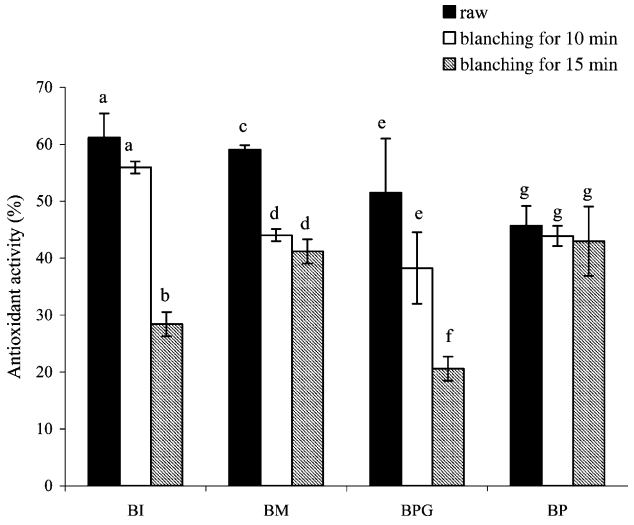

Fig. 4. Mean total antioxidant activity of spinach extracts. Antioxidant assay was measured using a β-carotene–linoleate model system. Data are means of three determinations. Different letters indicate significant difference at the level of $p < 0.05$ between raw and blanched samples of the respective spinach: BI, bayam itik; BM, bayam merah; BPG, bayam panjang; BP, bayam putih.

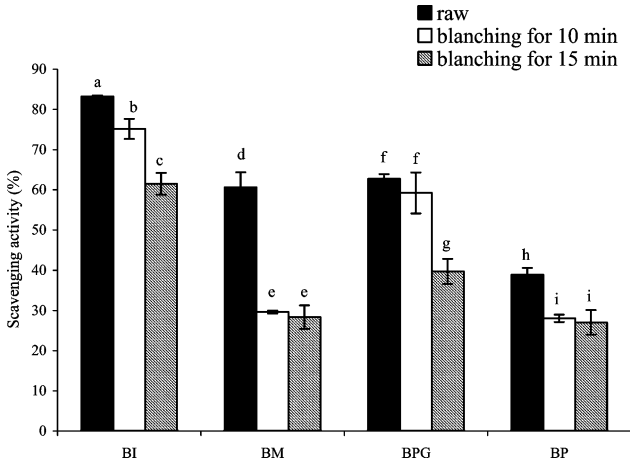

Fig. 5. Mean scavenging activity of spinach extracts. Scavenging activity was measured using the DPPH radical test. Data are means of three determinations. Different letters indicate significant difference at the level of $p < 0.05$ between raw and blanched samples of the respective spinach: BI, bayam itik; BM, bayam merah; BPG, bayam panjang; BP, bayam putih.

38 ± 6% and 43 ± 1%, respectively, after being blanched for 10 min. ANOVA showed no significant differences in the total antioxidant activities between raw and 10 min-blanched samples of BPG and BP. However, antioxidant activity of BM were reduced significantly ($p < 0.05$) after blanching for 10 min.

Mean total antioxidant activities of the 15 min blanched BI, BM, BPG and BP (28 ± 2%, 41 ± 2%, 21 ± 2% and 43 ± 2%, respectively) decreased significantly ($p < 0.05$) when compared with the raw ones, except for BP. The results of ANOVA analysis only revealed a significant decrease ($p < 0.05$) between the samples of BI and BPG blanched for 10 and 15 min (Fig. 4).

Antioxidant activity of all the studied spinach was in the order: raw > blanched for 10 min > blanched for 15 min.

3.2. Free radical-scavenging activity

The effect of blanching time on DPPH free radical-scavenging activity of the four spinach extracts is presented in Fig. 5. Among the studied spinach species, BI exhibited the greatest scavenging activity of DPPH free radical, followed by BPG, BM and BP (83 ± 1%, 63 ± 1%, 61 ± 2% and 39 ± 4%, respectively). Except between BPG and BM, there were significant differences ($p < 0.05$) in means of radical-scavenging activity among the spinach species. The free radical-scavenging activity of the studied raw spinach was in a similar order, as shown by the total antioxidant activity.

The radical-scavenging activities of BI, BM and BP showed significant decreases ($p < 0.05$) to 75 ± 2%, 29 ± 4% and 28 ± 1%, respectively after blanching for 10 min, except for BPG. As compared to the raw spinach, blanching for 15 min did show a significant decrease ($p < 0.05$) in scavenging activity (Fig. 5). Results from ANOVA analysis indicated that BI and BPG blanched for 10 min showed a significant difference ($p < 0.05$) in scavenging activity compared with the 15 min-blanched samples (Fig. 5). On the other hand, there was no significant difference in scavenging activity of blanched BP and BM between 10 and 15 min. DPPH radical-scavenging activity of the studied spinach was in the order: raw > blanched for 10 min > blanched for 15 min.

3.3. Total phenolic content

Fig. 6 shows the total phenolic content of raw and blanched spinach extracts. Among the studied spinach species, raw BM had the highest total phenolic content (107 ± 1.08 g/kg), followed by BP (101 ± 0.36 g/kg), BPG (85.6 ± 4.71 g/kg) and BI (69.4 ± 2.17 g/kg). ANOVA showed significant differences ($p < 0.05$) in total phenolic content among the studied raw spinach species.

Results show that the total phenolic content for all the spinach species, tends to decrease after blanching. After 5 min of blanching, BM lost the greatest amount of total phenolic content (51%), followed by BP (31%), BPG (8%) and BI (1%). In addition, BM lost a total of 71% total phenolic content after 15 min of blanching, followed by BPG (47%), BP (33%) and BI (9%).

One-way ANOVA analysis revealed a significance difference ($p < 0.05$) between raw and 10 min-blanched samples of BM, BP and BPG, except for BI. Furthermore, there was a significant decline ($p < 0.05$) in total phenolic content of all the studied raw spinach species

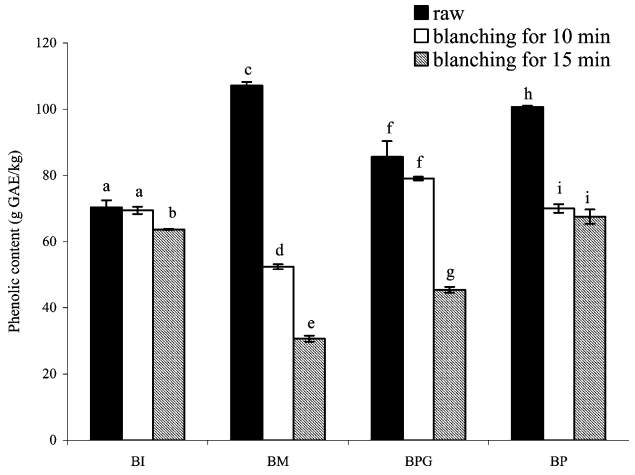

Fig. 6. Mean total phenolic contents of spinach extracts. Data are means of three determinations. Different letters indicate significant difference at the level of $p < 0.05$ between raw and blanched samples of the respective spinach: BI, bayam itik; BM, bayam merah; BPG, bayam panjang; BP, bayam putih.

after being blanched for 15 min. Among all the spinach species, only BP showed insignificant loss in total phenolic content between 15 and 10 min of blanching.

4. Discussion

Spinach has been reported as one of the many vegetables rich in antioxidant components (Cao, Sofic, & Prior, 1996; Gil, Ferreres, & Thomás-Barberán, 1999; Vinson, Hap, Su, & Zubik, 1998). Carotenoids, ascorbic acid, flavonoids and phenolic acids might be some of components able to contribute to the high antioxidant activity. The results revealed that the total antioxidant activity of BI was not significantly higher than the rest of the spinach, except for BP. This finding could be due to the antioxidant components present in spinach that contribute to a similar antioxidant activity. In this study, for example, extract of BM was more reddish than other spinach extracts. This colour is contributed by the red pigmentation in its leaves and stalks. In Malaysia, BM is the only red spinach. This red pigmentation could be one of the phenolic compounds. The colour of red vegetables is reported to be caused by anthocyanins (Prior et al., 1998). This has been shown by the total phenolic content of BM (Fig. 6). A study by Velioglu et al. (1998) found that red onion exhibited higher total phenolic content than other plants. On the other hand, Vinson et al. (1998) reported that beets had higher antioxidant activity than red onion. This could support our findings of no significant difference between phenolic contents of BM and BP. Besides colour pigmentation, other factors such as maturity and variety, can also influence the antioxidant capacity of vegetables (Prior et al., 1998). Romani et al. (2002) have indicated that a quantitative variation in phenolic compound content could be due to variety, different agronomic conditions, tissue type (red, green or white) and whether from outer or inner leaves.

The antioxidant activity in all types of raw spinach was higher than that of the blanched counterparts. Cooking practices, such as blanching, may greatly influence the loss of antioxidant components in leafy vegetables (Yadav & Sehgal, 1995). A loss of approximately 50% antioxidant activity is caused by blanching (Hunter & Fletcher, 2002). Findings showed that antioxidant activities of BPH, BI and BM decreased after blanching. This may be due to the blanching time and the temperature, that could reduce the antioxidant components and antioxidant activity.

Besides temperature, time is one of the critical factors affecting the loss of antioxidant components and antioxidant activity during blanching. About 82% of phenolic compounds were lost into the cooking water, after the green vegetables had been blanched for 15 min (Price, Bacon, & Rhodes, 1997). Chu et al. (2000) reported that blanching for less than 1 min would retain the high antioxidant activity in green leaves of sweet potatoes. Papetti, Daglia, and Gazzani (2002) reported a decrease in total antioxidant values when vegetables juices were cooked at 102 °C for 10 min. In addition, Gil et al. (1999) reported that boiling of fresh-cut spinach for 10 min released 50–60% of its antioxidant components into the cooking water. Hunter and Fletcher (2002) have indicated that boiling processes at above 95 °C would decompose the antioxidant components of vegetables. However, studies have shown that some of the antioxidant components in vegetables remain unchanged after cooking (Ewald, Fjelkner-Modig, Johansson, Sjöholm, & Åkesson, 1999).

The extraction medium used in this study might have influenced the extraction of phenolic compounds in the form of glycosides, or bound to the cell wall. For quantitative analysis, most workers have used aqueous methanol to obtain a more reliable result. However, in this study, water was preferred as an extraction medium because the regular household practice is to cook spinach in the presence of water.

The ability of spinach extract to act as a free radical-scavenger or hydrogen donor was revealed by DPPH radical-scavenging activity assay. Phenolic compounds have strong free radical-scavenging activity (Proteggente, Wisemen, van de Put, & Rice-Evans, 2003). DPPH radical-scavenging activity, for raw spinach samples, was seen to decrease after blanching. This may be due to losses or degradation of certain types of phenolic compounds or other DPPH free radical-scavenger components during blanching. This result supports Papetti et al. (2002) who reported that the radical-scavenging activity would decrease if the vegetables were exposed to heat, such as with blanching. According to Joubert (1990), blanching causes solubilization of phenolic compound and hence leads to loss of total phenolic compounds from the final

product. The study shows that spinach (*Amaranthus* sp.) contains water-soluble components that possess antioxidant activity, based on the two different assays. Blanching for up to 15 min may affect the antioxidant activity and phenolic content in raw spinach.

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References

Amin, I., Zamaliah, M. M., & Chin, W. F. (2004). Total antioxidant activity and phenolic content in selected vegetables. *Food Chemistry, 87*, 581–586.

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

Chu, Y. H., Chang, C. L., & Hsu, H. F. (2000). Flavonoid content of several vegetables and their antioxidant activity. *Journal of the Science of Food and Agriculture, 80*, 561–566.

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

Gil, M. I., Ferreres, F., & Thomás-Barberán, F. A. (1999). Effect of postharvest storage and processing on the antioxidant constituents (flavonoids and vitamin C) of fresh-cut spinach. *Journal of Agricultural and Food Chemistry, 47*, 2213–2217.

Hunter, K. J., & Fletcher, J. M. (2002). The antioxidant activity and composition of fresh, frozen, jarred and canned vegetables. *Innovative Food Science and Emerging Technology, 3*, 399–406.

Joubert, E. (1990). Effect of batch extraction conditions o extraction of polyphenols from rooibos tea (*Aspalathus linearis*). *International Journal of Food Science and Technology, 25*, 339–343.

Lee, J. H., Park, J. H., & Choi, J. S. (1996). The antioxidant activity of Ecklonia stolonifera. *Archieve Pharmaceutical Research, 19*(3), 223–227.

Papetti, A., Daglia, M., & Gazzani, G. (2002). Anti and prooxidant water soluble activity of *Chicorium* genus vegetables and effect of thermal treatment. *Journal of Agricultural and Food Chemistry, 50*, 4696–4704.

Price, K. R., Bacon, J. R., & Rhodes, M. J. C. (1997). Effect of storage and domestic processing on the content and composition of flavonol glucosides in onion (*Allium Cepa*). *Journal of Agricultural and Food Chemistry, 45*, 938–942.

Prior, R. L., Cao, G., Martin, A., Sofic, E., McEwen, J., O'Brien, C., et al. (1998). Antioxidant capacity as influenced by total phenolic and anthocyanin content, maturity, and variety of *Vaccinium* species. *Journal of Agricultural and Food Chemistry, 46*, 2686–2693.

Proteggente, A. R., Wisemen, S., van de Put, F. H. M. M., & Rice-Evans, C. A. (2003). The relationship between the phenolic composition and the antioxidant activity of fruits and vegetables. In C. M. Rice-Evans & L. Packer (Eds.), *Flavonoids in Health and Disease* (2nd ed.). New York: Marcel Dekker Inc.

Rimm, E. B., Ascherio, A., Grovannucci, E., Spielgelman, D., Stampfer, M. J., & Willett, W. C. (1996). Vegetable, fruit and cereal fiber intake and risk of coronory heart disease among men. *Journal of the American Medical Association, 275*, 447–451.

Romani, A., Pinelli, P., Galardi, C., Sani, G., Cimato, A., & Heimler, D. (2002). Polyphenols in greenhouse and open-air-grown lettuce. *Food Chemistry, 79*, 337–342.

Steinmetz, K. A., & Potter, J. D. (1996). Vegetables, fruits and cancer prevention: a review. *Journal of American Diet Association, 96*, 1027–1039.

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.

Vinson, J. A., Hap, Y., Su, X., & Zubik, L. (1998). Phenolic antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry, 46*, 3630–3634.

Wettasinghe, M., & Shahidi, F. (1999). Antioxidant and free radical-scavenging properties of ethanolic extracts of defatted borage (*Borago officinalis* L.) seeds. *Food Chemistry, 67*, 399–414.

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