International Food Research Journal 16: 45-51 (2009)

Effect of boiling and stir frying on total phenolics, carotenoids and radical scavenging activity of pumpkin (*Cucurbita moschato*)

*Azizah, A. H., Wee, K. C., Azizah, O. and Azizah, M.

Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 Serdang, Selangor, Malaysia

Abstract: Effect of various cooking methods on antioxidant content and radical scavenging activity of pumpkin was evaluated. Pumpkin (*Cucurbita moschata*) was boiled and stir-fried for 2, 4 and 6 minutes respectively. Beta-carotene and lycopene were determined using HPLC and total phenolics measured using Folin-Ciocalteu method. The free radical scavenging activity of the samples was determined using 1, 1-diphenyl-2-picrylhydrazyl assay. Interestingly, result of the study showed an increase in both beta-carotene (2 to 4 times) and lycopene (17 to 40 times) content of pumpkin after cooking for 2, 4 and 6 minutes. However, the treatment resulted in 18 to 54% losses of total phenolics content of the pumpkin. Nevertheless, the free radical scavenging activity exhibited by cooked pumpkins was found to be high, in the range of 81.1% to 94.6% with IC_{50} of 1.41 to 1.62 mg ml^{-1}.

Keywords: Antioxidant activity, beta-carotene, boiling, lycopene, pumpkin, stir-frying

Introduction

Plant-based foods are known to contain significant amounts of bioactive compounds that can provide desirable health benefits beyond basic nutrition and reduce the risk of degenerative diseases such as cancer and cardiovascular disease (Ames *et al*., 1993). Deep-coloured vegetables and fruits are known to be good sources of phenolics, including flavonoid and anthocyanin, and carotenoids (Qian *et al*., 2004; Sass-Kiss *et al*., 2005; Trappey *et al*., 2005; Cieslik, 2006). Steinmetz and Potter (1991) published the first comprehensive review about the relationship between vegetables, fruits and cancer. They concluded that consumption of both vegetables and fruits were associated with reduced risks of human cancers, especially cancers of the respiratory and gastrointestinal tract (lung, esophagus, colon and stomach). The protection against mutagenicity and cytotoxicity provided for by vegetables and fruits were believed to be attributed to the presence of various antioxidants that protect humans against oxidative damages by inhibiting or quenching free radicals and reactive oxygen species (Ikken *et al*., 1999).

Food preparation at home, in particular, cooking is often the final step in food processing. Most of the vegetables are commonly cooked either by simple boiling, microwave or stir-frying before consumed. These cooking processes would bring about a number of changes in physical characteristics as well as chemical composition of the vegetables. Many studies have shown that various cooking methods affected content of phytochemicals, in particular, antioxidants present in the vegetables (Ismail *et al*., 2004; Simone and Schlich, 2006; Turkmen *et al*., 2005; Zhang and Hamauzu, 2004). Podşedek (2007) suggested that both antioxidant levels and activities of processed vegetables were lower than those of the corresponding fresh samples. This is probably attributed to degradation of the bioactive compounds and absorption of water during boiling, resulting in dilution of the active compounds. On the other hand, Simone and Schlich (2006) reported significant increase in release of beta-carotene and tocopherol in broccoli upon cooking.

Pumpkin (*Cucurbita moschata)* is defined as fruit botanically although commonly regarded as vegetable in consumer terms. Flesh and seeds of pumpkin are commonly used for culinary and

*Corresponding author.
Email: *azizah@food.upm.edu.my*
Tel.: +603 8946 8374 ; Fax: +603 8942 3552

medicinal purposes. Carotenoids are the natural plant pigments responsible for the orange colour of pumpkin. Murkovic *et al*. (2002) reported that three species of pumpkin (*Cucurbita pepo, C. maxima and C. moschata)* consisted of beta-carotene (0.06-7.4 mg/100g, alpha-carotene (0-7.5 mg/100g) and lutein (0-17 mg/100g). Similarly, Amotz and Fishler (1998) reported that pumpkin consisted of both β-carotene and lycopene.

The aim of the present study is to evaluate degradation as well as release of the bioactive compounds (total phenolic, beta-carotene and lycopene) and free radical scavenging activity of pumpkin (*Cucurbita moschata*) as affected by different cooking (boiling and stir-frying) methods.

Materials and Methods

Plant material

Fresh pumpkin of commercial maturity was obtained from a supermarket in Selangor, Malaysia. Pumpkin used originated from Malaysia and of unknown variety. Pumpkin was randomly sampled from the shelf of the supermarket.

Preparation of samples

The fruits were carefully washed with tap water, dried with a soft cloth and the skin peeled. Skinless pumpkin was then cut into small pieces (1.5 cm x 1.5 cm x 1.5 cm) and divided into seven portions (10 g each). One portion was retained raw while others were cooked using methods specified.

Thermal treatment of samples

Ten g pumpkin was boiled in boiling water (100˚C) or stir-fried in hot oil (170˚C) for 2, 4 or 6 minutes respectively. The 2, 4, and 6 minutes were selected for stir-frying and boiling of the samples, as these are the times normally used when cooking pumpkin and similar fruits or vegetables. The samples were then drained and cooled before extraction process.

Preparation of the extract

Extraction was carried out according to the method of Skerget *et al*., (2005) with modification. Approximately 5 g sample was extracted using 50 ml ethanol in 250 ml conical flask. The flask was placed in a water bath at 25°C for 1hr. The crude extraxt was then filtered through a 0.4µm filter (Whatman, Maidstone, England) and the solvent was then

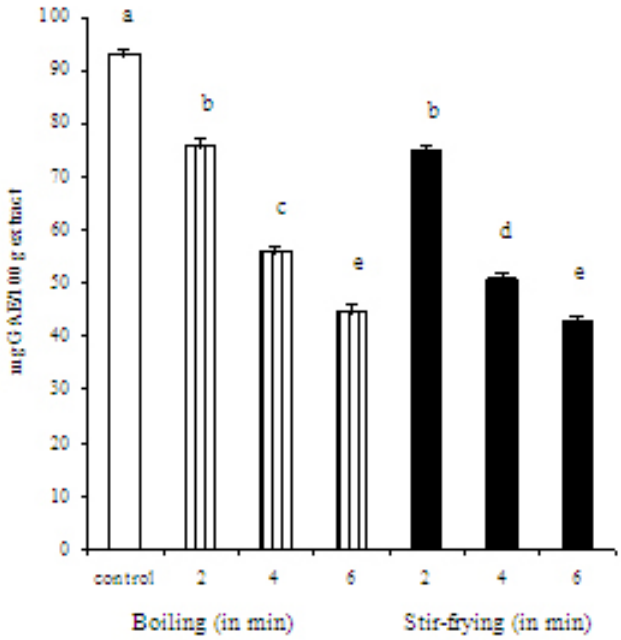

Figure 1. The total phenolic content of pumpkin before and after thermal treatment

Data represents mean of three independent analyses
[abc] Variation in the letters between samples indicates significance difference at 5% level ($P < 0.05$) utilizing vDuncan's test

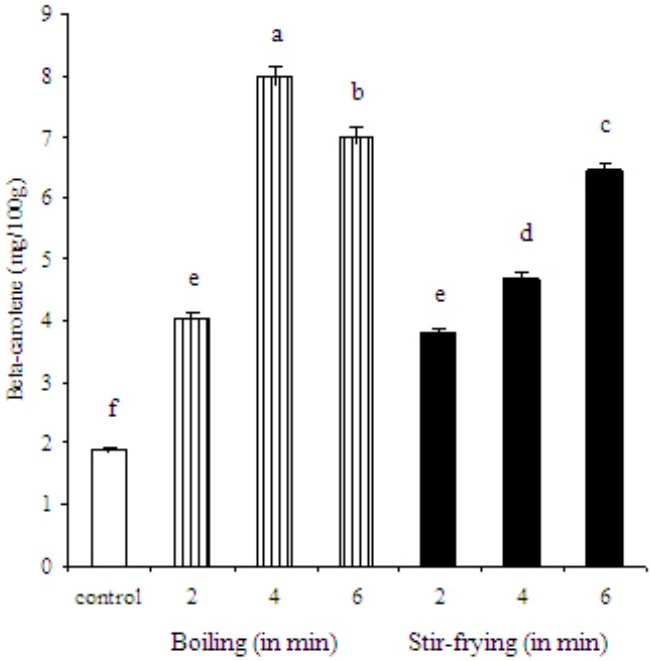

Figure 2. Beta-carotene content of pumpkin before and after thermal treatment

Data represents mean of three independent analyses
[abc] Variation in the letters between samples indicates significance difference at 5% level ($P < 0.05$) utilizing Duncan's test

evaporated off under vacuum. The extraction yield was then determined as weight %. The extracts were kept at -20°C until analyses.

Determination of phenolic compounds

Total phenolic content of the extracts was determined utilizing Folin-Ciocalteu assay system (AOCS, 1990). A 0.5 ml extract was added with 2.5 ml of Folin-Ciocalteu reagent followed by addition of 2 ml sodium carbonate (Na_2CO_3) (75 g/l). The sample was then incubated for 5 min at 50°C. The absorbance was then measured at 760 nm using Shimadzu UV-1650 PC Spectrophotometer (Kyoto, Japan). The results were then expressed as mg gallic acid equivalents per gram of extract (mg GAE/g) that was derived from a calibration curve.

Determination of carotenoid content

β-carotene and lycopene were determined according to the method of Tee and Lim (1991). A 5g sample was hydrolyzed with 20 ml of 95% (v/v) ethanol (HPLC grade) and 5 ml of 100% KOH, and refluxed for 30 min. The hydrolysate was then extracted using n-hexane and passed through anhydrous sodium sulphate for drying purposes. The extraction was repeated three times. Extracted samples were then filtered through 0.45µm nylon membrane filter (Whatman, Maidstone, England) and analysed using a reversed phase HPLC using µ Banda Pak C_{18} (3.9 x 300 mm) column and acetonitrile-methanol-ethyl acetate (88:10:2) as the mobile phase. The carotenoids eluted were detected and quantified using a UV-Visible detector attached to the 600 controller model HPLC (Waters, Milford, MA, USA). The retention time (t_r) and peak areas of appropriate standards (β-carotene and lycopene) were used to identify and quantify the isolated compounds.

Determination of antioxidant activity

The free radical scavenging activity of the samples was measured in accordance to the method of Brand-Williams (Brand-Williams *et al*., 1995) with modifications. The extracts were dissolved in 1.0 ml MeOH and the solutions added to a 1.0 ml DPPH· solution at room temperature. The absorbance at 515 nm was measured utilizing UV-1601 Shimadzu spectrophotometer. The results were expressed as percentage of reduction of the initial DPPH· absorption by test samples as follows:

$$\text{DPPH scavenging effect (\%)} = [(A0 - At) / A0] \times 100$$

Where A_0 is the absorbance of the control at t = 0 min, and A_t is the absorbance of the antioxidant at t = 15 min.

Table 1. Effect of cooking on radical scavenging activity and IC_{50} of pumpkin

	Antioxidant Activity (%)	IC50 (mg ml-1)
Fresh	78.4 ± 1.7^{d}	1.91
Boil 2 minutes	89.2 ± 1.4^{ab}	1.41
Boil 4 minutes	94.6 ± 1.9^{a}	1.56
Boil 6 minutes	91.9 ± 1.9^{ab}	1.57
Stir-fry 2 minutes	81.2 ± 1.8^{cd}	1.58
Stir-fry 4 minutes	89.2 ± 2.2^{ab}	1.59
Stir-fry 6 minutes	86.5 ± 1.6^{bc}	1.62

Each mean represents analyses of three independent samples
abc Variation in the following letters between samples indicates significant of difference by Duncan's test at 5% level ($P < 0.05$)

The IC_{50} is defined as the concentration of antioxidant necessary to decrease the initial DPPH concentration by 50%. The IC_{50} of the samples was derived from the % scavenging activity vs. concentration plot and is expressed as mg/ml.

Statistical analysis

All data was reported as mean ± standard error of triplicate determinations and analyzed using one-way analysis of variance (ANOVA) with significant differences between means determined at $p<0.05$, measured with Duncan's multiple range tests using Statistical Package for Social Science Research version 14 (SPSS).

Results and Discussion

The effect of cooking on bioactive compounds of pumpkin

The study reveals the stability as well as degradation of antioxidants present in the samples. The total phenolic content of raw and cooked pumpkins is shown in Figure 1. As expected, thermally treated pumpkin for 2, 4 and 6 min. resulted in significant losses (18.3% to 53.8%) of total phenolic compounds. Similar trend was observed for both boiled and stir-fried pumpkin. The longer the cooking time, the greater losses of the total phenolic compound measured. This could be due to breakdown of phenolics or losses (leached out) during cooking as most of the bioactive compounds are relatively unstable to heat and easily solubilized (Crozier *et al*., 1997; Zhang and Hamauzu, 2004). Turkmen *et al*. (2005) reported that after cooking (boiling, steaming and microwaving), the total phenolic compound of squash, peas and leek was significantly ($p<0.05$) reduced. In a study carried out by Ismail *et al*. (2004), spinach was found to have the highest phenolic content, followed by swamp cabbage, kale, shallots and cabbage. Blanching for 1 min in boiling water reduced (12-26%) total phenolics in these vegetables.

Similarly, Sahlin *et al*. (2004) found that cooking by boiling, baking and frying resulted in a significant ($p<0.01$) reduction in the total phenolic and ascorbic acid content of tomatoes. On the other hand, in the same study, they found that cooking gave rise to an increase in phenolics in green beans, pepper and broccoli. The authors suggested that this is probably due to the increased level of free flavonols in the vegetables as affected by the heat treatment.

Beta-carotene and lycopene content of raw and cooked pumpkin are presented in Figure 2 and 3 respectively. Interestingly, results from the study showed that both boiling and stir-frying increased the β-carotene (2 to 4.2 times) and lycopene (17 to 40 times) content of pumpkin. Boiling for 4 minutes resulted in the highest (4.2 times) increase of β-carotene to 8.0 mg/100g. Six minutes boiling, on the other hand, resulted in 270% (3.7 times) increase in β-carotene content. Similarly, boiling for 4 and 6 min. resulted in higher lycopene content of pumpkin by 37 and 40 times respectively. Results from this study revealed that thermal treatment enhanced availability of the carotenoid. Similar results have been reported by previous researchers (Stahl and Sies, 1992; Rock *et al*., 1998). It has been reported that there is an enhanced bioavailability of carotenoids after heat treatment in carrots and spinach (Rock *et al*., 1998). Stahl and Sies (1992) reported that bioavailability of lycopene and β-carotene from cooked tomatoes and carrots was higher than that of

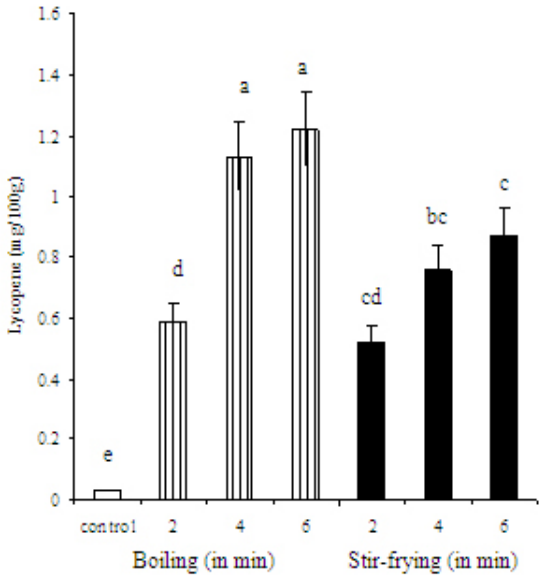

Figure 3. Lycopene content of pumpkin before and after thermal treatment

Data represents mean of three independent analyses

[abc] Variation in the letters between samples indicates significance difference at 5% level ($P < 0.05$) utilizing Duncan's test

raw samples. However, boiling for 6 minutes have resulted in significantly ($p<0.05$) lower β-carotene content than that of 4 minutes boiling. This may be due to the prolonged boiling time that may result in longer exposure of the pumpkin to heat, oxygen and light. Similar trend can be seen when pumpkin was stir-fried for 2-6 minutes, which resulted in 2 to 3.4 times increase in β-carotene content and 17 to 29 times increase in lycopene content.

Results of the study revealed that boiling resulted in higher carotenoids content compared to that of stir-frying. This is probably due to the fact that carotenoids are fat-soluble compounds and solubilized readily in oil during stir-frying, and thus resulted in decrease of its contents in pumpkin. In addition, pumpkin was exposed to a much lower temperature during boiling (100˚C) compared to that of stir frying (180˚C).

Studies have shown that thermally processed foods, especially fruits and vegetables, exhibited higher biological activities due to various chemical changes undergone during heat treatment (Kim *et al*., 2000; Dewanto *et al*., 2002). In general, processing of vegetables resulted in break down of the cellulose structure of the plant cell and thus improves the bioavailability of carotenoids (Van het Hof *et al*., 2000).

Effect of cooking on antioxidant activity of pumpkin

DPPH, a paramagnetic compound with an odd electron, shows strong absorption band at 517 nm in methanol. The absorbance decreases as a result of color change from purple to yellow due to the scavenging of free radical by antioxidants through donation of hydrogen to form the stable DPPH–H molecule (Chandrasekar *et al*., 2006). All the fresh and cooked pumpkins were able to reduce the stable, purple-coloured radical, DPPH, into the yellow-coloured DPPH-H reaching 50% of reduction. The IC_{50} values of the samples are shown in Table 1. Fresh and cooked pumpkin showed a small but significant difference of their antioxidant activity. Studies showed that the IC_{50} of boiled pumpkin at 2, 4, 6 minutes are found to be 1.41, 1.56 and 1.57 mg ml^{-1} respectively. On the other hand, stir-fried pumpkin revealed a small but significantly different of their IC_{50} after 2, 4 and 6 minutes frying respectively . The antioxidant activity demonstrated by the cooked pumpkin were found to decrease in the following order of boiled 2 minutes > boiled 4 minutes > boiled 6 minutes > stir-fried 2 minutes > stir-fried 4 minutes > stir-fried 6 minutes > fresh pumpkin.

Gahler *et al*. (2003) reported an improvement in the antioxidant activity of tomatoes after heat treatment

due to the increased release of phytochemicals, such as lycopene, from the matrix. Boiling of several vegetables would attribute to the suppression of oxidation by antioxidants due to thermal inactivation of oxidative enzymes (Yamaguchi *et al*., 2001). In addition the boiling process may destruct the cell wall and subcellular compartments thus release of potent radical-scavenging antioxidants. Turkmen *et al*. (2005) reported that boiling, microwave cooking and steaming induced significant increases in total antioxidant activity of pepper, green beans, broccoli and spinach.

The IC_{50} of boiled pumpkin found in this study was lower than that reported by Mau *et al*. (2004), who found that the IC_{50} of *Antrodia camphorata* mycelia was in the range of 1.70 to 2.06 mg ml^{-1} for white and red mycelia respectively. *A. camphorata* was reported to be commonly used as an antidote, anticancer, anti-itching and hepatoprotective drug (Mau *et al*., 2004). On the other hand, Chandrasekar *et al*. (2006) revealed that some polyherbal formulation, including Liv.52, New Livfit, Livina and Livomyn consisting of combinations of herbal extracts showed IC_{50} in the range of 24.1 to 186.9 μg ml^{-1}.

Conclusion

Results from the study showed that cooking affected both the bioactive compounds and their antioxidant activity. Boiling and stir frying pumpkin for 2, 4 and 6 min resulted in 18 to 45% degradation of its total phenolic compounds. However, interestingly, content of both beta-carotene and lycopene was found to increase 2.0 to 40.6 times upon boiling and stir-frying. The pumpkin boiled for 2 minutes have shown to have strong antioxidant activity. The evaluation of those bioactive compounds in pumpkin would provide information about the changes of phenolic compounds, carotenoids and their antioxidant activity during cooking.

Acknowledgements

The authors would like to thank the Ministry of Science, Technology and Innovation of Malaysia for financing the project and the Faculty of Food Science and Technology, Universiti Putra Malaysia, for the laboratory facilities.

References

Ames, B. M., Shigena, M. K. and Hagen, T. M. 1993. Oxidants, antioxidants and the degenerative diseases of aging. Proceedings of the National of Science of the United States America 90: 7915-7922

AOCS. 1990. In: Official methods and recommended practices of the American Oil Chemicsts' Society (4th ed.). Champaign: American Oil Chemists' Society.

Ben-amotz, A. and Fishler, R. 1998. Analysis of carotenoids with emphasis on 9-cis β-carotene in vegetables and fruits commonly consumed in Israel. Food Chemistry 62(4): 515-520.

Brand-Williams, W., Cuvelier, M. E. and Berset, C. 1995. Use of a free radical method to evaluate antioxidant activity. Lebensmittel-Wissenschaft and Technologie 28: 25-30.

Chandrasekar, D., Madhusudhana, K., Ramakrishna, S. and Diwan, P.V. 2006. Determination of DPPH free radical scavenging activity by reversed-phase HPLC: A sensitive screening method for polyherbal formulations. Journal of Pharmaceutical and Biomedical Analysis 40: 460–464.

Cieslik, E., Greda, A. and Adamus, W. 2006. Contents of polyphenols in fruit and vegetables. Food Chemistry 94: 135–142.

Crozier, A., Lean, M.E.J., McDonald, M.S. and Black, C. 1997. Quantitative analysis of the flavonoids content of commercial tomatoes, onions, lettuce and celery. Journal of Agricultural and Food Chemistry 45: 590-595.

Dewanto, V., Wu, X., Adam, K. K. and Liu, R.H. 2000. Thermal processing enhances the nutritional value of tomatoes by increasing total antioxidant activity. Journal of Agricultural and Food Chemistry 50: 3010–3014.

Gazzani, G., Papetti, A., Massolini, G. and Daglia, M. 1998. Anti- and proxidant activity of water soluble components of some common diet vegetables and effect of thermal treatment. Journal of Agricultural and Food Chemistry, 46: 4118-4122.

Gahler, S., Otto, K. and Bohm, V. 2003. Alterations of vitamin C, total phenolics, and antioxidant capacity as affected by processing tomatoes to different products. Journal of Agriculture and Food Chemistry 51: 7962–7968.

Ismail, A., Marjan, Z. M. and Foong, C.W. 2004. Total antioxidant activity and phenolic content in selected vegetables. Food Chemistry 87: 581-586.

Ikken, Y., Morales, P., Martinez, A., Marin, M.L., Haza, A.I., and Cambero, M.I. 1999. Antimutagenig effects of fruit and vegetables ethanolic extracts against N-nitrosamines evaluated by the Ames test. Journal of Agricultural and Food Chemistry 47: 3257-3264.

Kim, W.Y., Kim, J.M., Han, S.B., Lee, S.K., Kim, N.D., Park, M K. 2000. Steaming of ginseng at high temperature enhances biological activity. Journal of Natural Products 63: 1702–1704.

Mau J-L., Huang P-N., Huang S-J. and Chen C-C. 2004. Antioxidant properties of methanolic extracts from two kinds of Antrodia camphorata mycelia. Food Chemistry 86: 25-31.

Murkovic, M., Mulleder, U. and Neunteufl, H. 2002. Carotenoid content in different varieties of pumpkins. Journal of Food Composition and Analysis 15: 633-638.

Pinheiro Sant'Ana, H. M., Stringheta, P. C., Cardoso Brandão, S. C. and Cordeiro de Azeredo, R. M. 1998. Carotenoid retention and vitamin A value in carrot Daucus carota L. prepared by food service. Food Chemistry 61: 145–151.

Podşedek, A. 2007. Natural antioxidants and antioxidant capacity of Brasicca vegetables: A review. Lebensmittel-Wissenschaft and Technologie 40: 1-11.

Qian, J.-Y., Liu, D. and Huang, A.-G. 2004. The efficiency of flavonoids in polar extracts of Lycium chinense Mill fruits as free radical scavenger. Food Chemistry 87: 283–288.

Rock, C. L., Lovalvo, J. L., Emenhise, C., Ruffin, M. T., Flatt, S. W. and Schwartz, S. J. 1998. Bioavailability of b-carotene is lower in raw rather than in processed carrots and spinach in women. Journal of Nutrition 128: 913–916.

Sahlin, E., Savage, G.P. and Lister, C.E. 2004. Investigation of the antioxidant properties of tomatoes after processing. Journal of Food composition and Analysis 17: 635-647.

Sass-Kiss, A., Kiss, J., Milotay, P., Kerek, M. M. and Toth-Markus, M. 2005. Differences in anthocyanin and carotenoid content of fruits and vegetables. Food Research International 38: 1023–1029.

Skerget, M., Kotnik, P., Hadolin, M., Hras, A., Simonic, M. and Knez, Z. 2005. Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. Food Chemistry 89: 191-198.

Stahl, W. and Sies, H. 1992. Uptake of lycopene and its geometrical isomers is greater from heat-processed than from unprocessed tomato juice in humans. Journal of Nutrition 122: 2161–2166.

Steinmetz, K.A. and Potter, J.D. 1991. Vetables, fruits and cancer. I. Epidemiology Cancer Causes Control 2: 325-357.

Tee, E.S. and Lim, C.L. 1991. The analysis of carotenoids and retinoids: a review. Food Chemistry 41(2): 147-193.

Trappey, A., II, Bawadi, H.A., Bansode, R.R. and Losso, J.N. 2005. Anthocyanin profile of mayhaw (Cretaegus opaca). Food Chemistry 91: 665–671.

Turkmen, N., Sari, F. and Velioglu, S. 2005. The effect of cooking methods on total phenolics and antioxidant activity of selected green vegetables. Food Chemistry 93: 713-718.

Van het Hof, K.H., de Boer, B.C.J., Tijburg, L.B.M., Lucius, B.R.H.M., Zijp, I., West, C.E., Hautvast, J.G.A.J. and Weststrate J.A. 2000. Carotenoid bioavailability in humans from tomatoes processed in different ways determined from the carotenoid response in the triglyceride-rich lipoprotein fraction of plasma after a single consumption and in plasma after four days of consumption. Journal of Nutrition 130: 1189–1196.

Yamaguchi, T., Mizobuchi, T., Kajikawa, R., Kawashima, H., Miyabe, F., Terao, J.
2001. Radical-scavenging activity of vegetables and the effect of cooking on their activity. Food Science and Technology Research 7(3): 250–257.

Zhang, D. and Hamauzu, Y. 2004. Phenolics, ascorbic acid, carotenoids and antioxidant activity of broccoli and their changes during conventional and microwave cooking. Food Chemistry 88: 503-509.