Available online at www.sciencedirect.com
SCIENCE DIRECT®
Food Chemistry 87 (2004) 581–586
Food Chemistry
www.elsevier.com/locate/foodchem

Total antioxidant activity and phenolic content in selected vegetables

Amin Ismail *, Zamaliah M. Marjan, Chin W. Foong

Department of Nutrition and Health Sciences, Faculty of Medicine and Health Sciences, Universiti Putra Malaysia, Serdang, 43400 Selangor, Malaysia

Received 18 July 2003; received in revised form 8 January 2004; accepted 8 January 2004

Abstract

This study was carried out to determine the total antioxidant activity and phenolic content of selected common vegetables. The effect of thermal treatment on antioxidant activity and phenolic content were also studied. Kale, spinach, cabbage, swamp cabbage and shallots were used in this study. Among all the vegetables (fresh and thermally treated), shallots showed the highest total antioxidant activity followed by spinach, swamp cabbage, cabbage and kale. Spinach had an exceptionally high total phenolic content, followed by swamp cabbage, kale, shallots and cabbage. Except for shallots and cabbage, the antioxidant activities of kale, spinach and swamp cabbage were significantly decreased ($p < 0.05$) after thermal treatment. Moreover, this study revealed that a 1-min thermal treatment significantly decreased ($p < 0.05$) the total phenolic content of all vegetables studied.

Keywords: Total antioxidant activity; Total phenolic content; Vegetables

1. Introduction

Fruits and vegetables account for a small part of our daily caloric intake; however their benefits to health surpass their caloric contribution. The contributory factors are due to the presence of vitamins and provitamins, such as ascorbic acid, tocopherols and carotenoids and, in addition to that, they are also rich in a wide variety of phenolic substances (Loliger, 1991). Phenolic substances are a category of phytonutrients that exert strong antioxidant properties (Ho, 1992). They can be classified into simple phenols, phenolic acids, hydroxycinnamic acid derivatives and flavonoids. The ability of some of the phenolic substances to act as potent antioxidant components has been reported (Velioglu, Mazza, Gao, & Oomah, 1998; Kähkönen et al., 1999).

The principle function of antioxidants is in delaying the oxidation of other molecules by inhibiting the initiation or propagation of oxidizing chain reactions by free radicals and they may reduce oxidative damage to the human body (Namiki, 1990). The occurrence of such oxidative damage may be a significant causative factor in the development of many chronic diseases, such as cancer and cardiovascular diseases (Lindley, 1998; Papas, 1999). Several epidemiological studies have shown a negative association between intake of fruits and vegetables and certain diseases (Papas, 1999).

Cao, Sofic, and Prior (1996) found that vegetables, such as kale, beets, pepper, broccoli, spinach, shallots, potato, carrots and cabbage, had high antioxidant activities. Kale, spinach, swamp cabbage, cabbage and shallots are some of the commonly consumed vegetables in Malaysia. Beside antioxidant nutrients such as ascorbic acid, tocopherols, and carotenoids, these vegetables are also a good source of polyphenol components. Generally, Malaysians consume vegetables such as spinach, kale, and swamp cabbage as cooked food. Cooking may affect the antioxidant content in vegetables, especially components such as tocopherol, carotenoids, ascorbic acid and polyphenols. Their contribution as source food antioxidants can further be substantiated if more studies are done on their potential. Thus, it is the purpose of this study to determine the total antioxidant activity and phenolic content of fresh and thermally treated vegetables.

* Corresponding author. Tel.: +603-8946-8557; fax: +603-8942-6769.
E-mail address: amin@medic.upm.edu.my (A. Ismail).

0308-8146/$ - see front matter © 2004 Elsevier Ltd. All rights reserved.
doi:10.1016/j.foodchem.2004.01.010

2. Materials and methods

2.1. Vegetables

2.1.1. General

Five types of vegetables, namely kale (*Brassica alboglabra*), spinach (*Amaranthus sp.*), cabbage (*Brassica oleracea*), shallot (bulb part) (*Allium cepa*) and swamp cabbage (*Ipomoea reptans*), were purchased from several wet markets at Sri Serdang, Selangor, Malaysia. The vegetables (1.0 kg each) were randomly sampled from the shelf.

2.1.2. Chemicals

Linoleic acid, β-carotene, Tween 20 and ferulic acid were purchased from Sigma Chemical Co. (St Louis, MO, USA). Other chemicals used were of analytical grade.

2.1.3. Preparation of sample

One kilogramme of fresh vegetable was cleaned and washed under running tap water. For thermal treatment, 300 g edible portions of the vegetables were boiled for 1 min in 500 ml water. Then, excessive water was dripped off and the vegetables were air-dried under the fan. 100 g of sample was cut into small pieces and homogenised using a wet blender (National; model: MX-291N) for 2 min. The homogenised sample was transferred into an air-tight container and kept at −20 °C before the preparation of extract.

2.1.4. Extraction of sample

The homogenised sample was weighed and transferred to a beaker. Then, 70% (v/v) ethanol was added into it in the ratio of 1:5 and stirred at 200 rpm in an orbital shaker (Unimax 1010, Heidolph Instruments GmbH & Co. KG, Germany) for 1 h at room temperature. The extract was then separated from the residue by filtration through Whatman No. 1 filter paper. The remaining residue was re-extracted twice, and then the two extracts were combined. The residual solvent of ethanolic extract was removed under reduced pressure at 40 °C using a rotary evaporator (Laborata 4000; Heidolph Instruments GmbH & Co. KG, Germany). The ethanolic extract was produced in duplicates. The same extraction procedure using 70% (v/v) ethanol as an extraction medium was carried out on all the vegetables for the determination of total antioxidant activity and phenolic content.

2.2. Determination of total antioxidant activity

Antioxidant activity of vegetables extract was measured according to the method described by Amin and Tan (2002). One millilitre of β-carotene solution (0.2 mg/ml chloroform) was pipetted into a round-bottom flask (50 ml) containing 0.02 ml of linoleic acid and 0.2 ml of 100% Tween 20. The mixture was then evaporated at 40 °C for 10 min by means of a rotary evaporator to remove chloroform. After evaporation, the mixture was immediately diluted with 100 ml of distilled water. The distilled water was added slowly to the mixture and agitated vigorously to form an emulsion.

Five millilitres aliquots of the emulsion were transferred into different test tubes containing 0.2 ml of samples in 70% ethanol at a final concentration of 1 mg/ml. The tubes were then gently shaken and placed at 45 °C in a water bath for 2 h. The absorbance of the samples was measured at 470 nm using a Spectronic® Genesys™ 5 spectrophotometer (Milton Roy Company, New York) at initial time ($t = 0$) against a blank, consisting of an emulsion without β-carotene. Standards of the same concentration as samples were used for comparison; 0.2 ml of 70% ethanol in 5 ml of the above emulsion was used as the control. The measurement was carried out at 15 min intervals for 120 min. All samples were assayed in triplicate.

The antioxidant activity (AA) was measured in terms of successful bleaching of β-carotene by using the following equation.

$$\mathrm{AA} = \left(1 - \frac{(A_0 - A_t)}{(A^\circ_0 - A^\circ_t)}\right) \times 100,$$

where A_0 and A°_0 are the absorbance values measured at the initial incubation time 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.

2.3. Determination of total phenolic content

The amount of total phenolic was determined according to the method of Velioglu et al. (1998) which used Folin-Ciocalteu reagent. Extract was prepared at a concentration of 1 mg/ml. 100 μl of extract was transferred into a test tube and 0.75 ml of Folin-Ciocalteu reagent (previously diluted 10-fold with deionised water) were added and mixed. The mixture was allowed to stand at room temperature for 5 min. 0.75 ml of 6% (w/v) sodium carbonate was added to the mixture and then mixed gently. After standing at room temperature for 90 min, the absorbance was read at 725 nm using a UV–Vis spectrophotometer. The standard calibration (0.01–0.05 mg/ml) curve was plotted using ferulic acid. The total phenolic content was expressed as ferulic acid equivalents in milligrammes per 100 g vegetable extract.

2.4. Statistical analysis

The results obtained were analysed using one-way ANOVA for mean differences among vegetables. The independent *t*-test was used to analyse differences be-

tween fresh and thermally treatment vegetables. A Statistical Package for Social Science for Windows version 10.01 was used to analyse the data.

3. Results and discussion

3.1. Total antioxidant activity

The total antioxidant activity, which reflected the ability of the vegetable extracts to inhibit the bleaching of β-carotene, was measured and compared with that of the control which contained no antioxidant component. The β-carotene bleaching rates of the vegetable extracts are shown in Figs. 1 and 2. There was a decrease in absorbance values of β-carotene in the absence of vegetable extracts due to the oxidation of β-carotene and linoleic acid. The high absorbance values indicated that vegetable extracts possessed antioxidant activity.

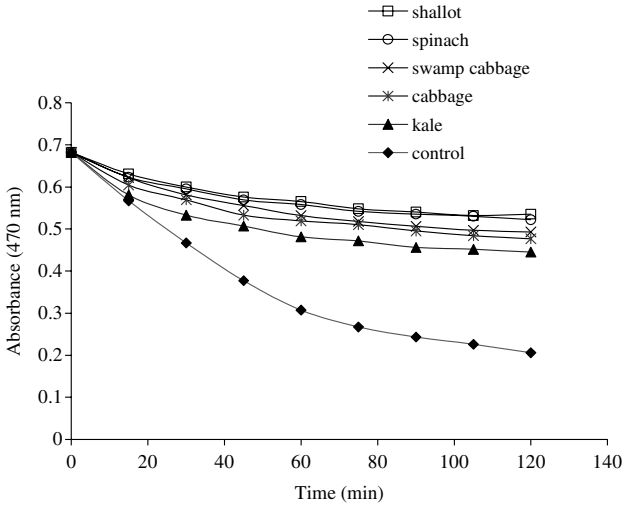

Fig. 1. Antioxidant activity of fresh vegetable extracts at 1 mg/ml using β-carotene-linoleate system.

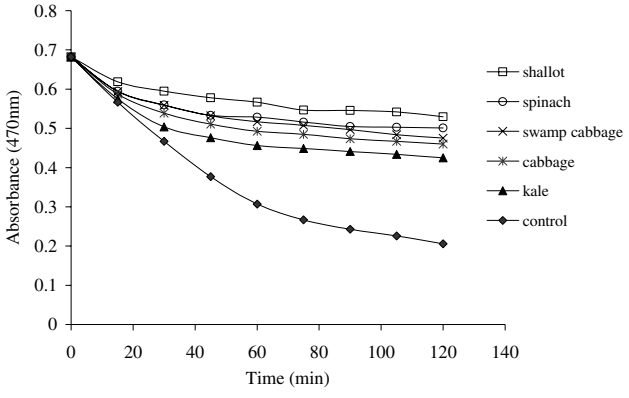

Fig. 2. Antioxidant activity of thermally treated vegetable extracts at 1 mg/ml using β-carotene-linoleate system.

The absorbance value of the control was significantly lower ($p < 0.05$) than the vegetable extracts. All the vegetable extracts showed similar trends with a significant decrease ($p < 0.05$) of absorbance value until 45 min. This indicated that the samples, whether fresh or thermally treated, had acted as effective antioxidants in the β-carotene-linoleate system, which inhibited the oxidation activity of β-carotene (Figs. 1 and 2). Among the studied vegetables, shallot exhibited the greatest inhibition of β-carotene bleaching rates, followed by spinach, swamp cabbage, cabbage and kale.

The comparison of mean total antioxidant activity of fresh and thermally treated vegetables is presented in a descending order in Fig. 3. Mean antioxidant activity of shallots, spinach, swamp cabbage, cabbage and kale were $69.1 \pm 1.1\%$, $66.4 \pm 1.1\%$, $60.3 \pm 1.0\%$, $59.3 \pm 4.1\%$ and $50.2 \pm 1.3\%$, respectively. Results of ANOVA analysis indicated that antioxidant activity of shallots is significantly higher ($p < 0.05$) than the rest of the fresh vegetables, except spinach. As shown in Fig. 3, the mean antioxidant activities of the 1-min boiled vegetables were similar to the fresh vegetable, shallots > spinach > swamp cabbage > cabbage > kale ($68.5 \pm 2.4\%$, $61.9 \pm 0.6\%$, $56.5 \pm 1.1\%$, $53.4 \pm 0.6\%$ and $45.9 \pm 1.3\%$, respectively).

In a study carried out by Cao et al. (1996) on antioxidant activity, using oxygen radical absorbance capacity assay, kale was found to have the highest antioxidant activity, followed by spinach and cabbage. Kurilich et al. (1999) reported that kale had higher levels of vitamins (α-carotene, β-carotene, α-tocopherol, γ-tocopherol, and ascorbate) than did cabbage. The differences of the results obtained from this study compared

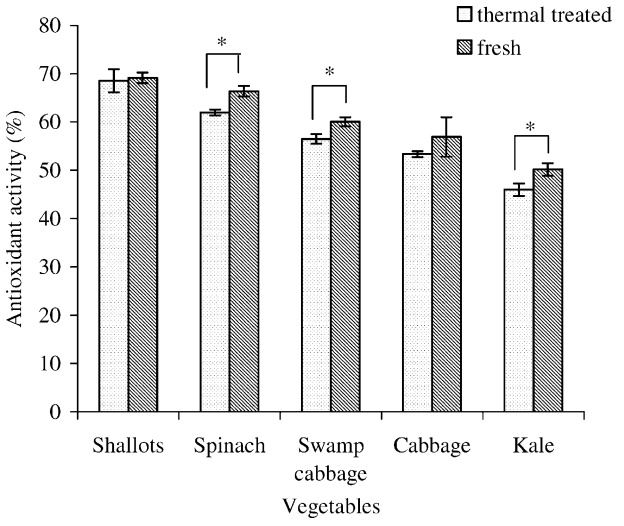

Fig. 3. Mean total antioxidant activity of vegetable extracts. Asterisk (*) indicates a significant difference at the level $p < 0.05$ between fresh and thermally treated vegetables. Antioxidant activity was measured using a β-carotene-linoleate system. Results are means of three determinations. Values indicate that the coefficient of variation was less than 7%.

to the previous findings may have been due to the differences in species cultivation of the kale used and differences in the extraction methods. Environmental factors, such as climatic growth conditions, growth, ripening stage, temperature, duration of storage and thermal treatment may have influenced the antioxidant activity (Gazzani, Papetti, Massolini, & Daglia, 1998).

Although the amounts of antioxidant vitamins in shallot bulb were lower than other vegetables (Puwastein, Burlingame, Raroengwichit, & Sungpuag, 2000), they had the highest antioxidant activity. Therefore, shallots may have some powerful antioxidant compounds other than α-tocopherol, carotenoids and vitamin C. According to Shahidi and Wanasundara (1992), shallots had high flavonoid contents which may have contributed to their high antioxidant activity. A high antioxidant activity of spinach and swamp cabbage may be attributed to the nutrient antioxidants found in these vegetables. According to Tee, Lim, Chong, and Khor (1996), swamp cabbage and spinach have high contents of carotenoids, ascorbic acid and α-tocopherol and these might have been the main factors that contribute to the high antioxidant activity. The low antioxidant activity of cabbage was in agreement with Cao et al. (1996).

Antioxidant activity of vegetable extracts also depends on the type and polarity of the extracting solvent, the isolation procedures and purity of active compounds, as well as the assay techniques and substrate used (Meyer, Heinonen, & Frankel, 1998). In the study of Tsuda et al. (1994), less polar solvents provided slightly more active extracts than mixtures with ethanol or methanol, or methanol alone for tamarind seed coats. The extraction solvent in this study was 70% ethanol (v/v) while 100% acetone was used by Cao et al. (1996). This factor may also have affected the results in the different findings.

Thermal treatment has been known to affect antioxidant activity (Gazzani et al., 1998). Antioxidant activity of kale, spinach and swamp cabbage were reduced significantly ($p < 0.05$) after 1 min of thermal treatment. The thermal treatment on cabbage and shallots in this study did not show any significant difference in antioxidant activity (Fig. 3). However, increasing boiling time may have affected the antioxidant activity of these vegetables. Yadav and Sehgal (1995) showed that cooking spinach in an open pan for 30 min had reduced the vitamin C content by 90% while the β-carotene content was reduced down to 50%. Microwave cooking of kale for 6 min, in a small amount of water, produced a loss of 15–57% of the xanthophylls and 15% loss of the carotenoids (Micozzi, Beecher, Taylor, & Khachik, 1990).

3.2. Total phenolic content

The total phenolic content of the vegetable extracts is shown in Fig. 4. Among all the fresh vegetables, spinach had the highest phenolic content (7167 ± 73 mg/100 g vegetable extract), followed by swamp cabbage (4175 ± 41 mg/100 g), kale (3689 ± 66 mg/100 g), shallots (2528 ± 43 mg/100 g) and cabbage (1107 ± 57 mg/100 g). For thermally treated vegetables, spinach (6168 ± 41 mg/100 g) still had an exceptionally high total phenolic content, followed by kale (3251 ± 123 mg/100 g), swamp cabbage (3095 ± 74 mg/100 g), shallots (2187 ± 49 mg/100 g) and cabbage (886 ± 52 mg/100 g).

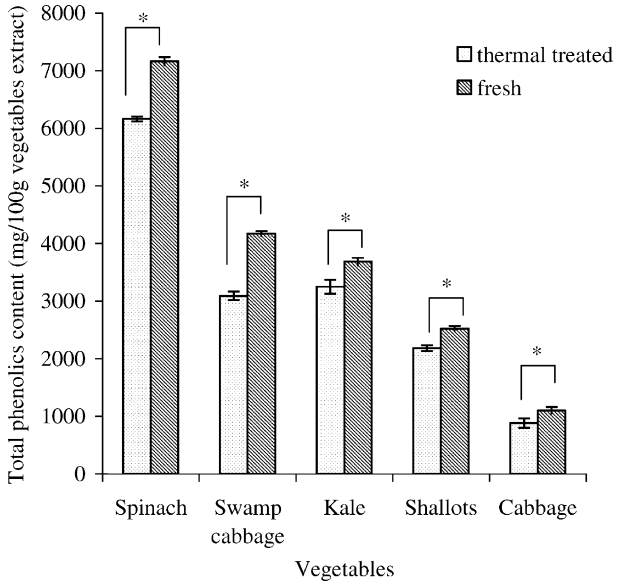

Fig. 4. Mean total phenolic content of vegetable extracts. Asterisk (*) indicates a significant difference at the level $p < 0.05$ between fresh and thermally treated vegetables. Results are means of three determinations. Values indicate that the coefficient of variation was less than 9%.

Swamp cabbage lost the highest amount of phenolic content (26%), followed by cabbage (20%), spinach (14%), shallots (13%) and kale (12%) after a 1-min blanching in boiling water. ANOVA showed significant differences ($p < 0.05$) in the total phenolic content between fresh and treated vegetables (Fig. 4). The findings indicated that phenolic compounds were very sensitive to heat treatment even in a short period of cooking.

Kähkönen et al. (1999) found that the total phenolic contents of vegetables were very low compared to fruits. Vinson, Hap, Su, and Zubik (1998) found that beets had the highest total phenolic content, followed by red onion, broccoli and kidney beans. Meanwhile, Velioglu et al. (1998) reported that red onion had a higher total phenolic content than other plant materials.

Several studies have reported on the relationships between phenolic content and antioxidant activity. Some authors found a correlation between the phenolic content and the antioxidant activity, while others found no such relationship. Velioglu et al. (1998) reported a strong relationship between total phenolic content and antioxidant activity in selected fruits, vegetables and grain products. No correlation between antioxidant ac-

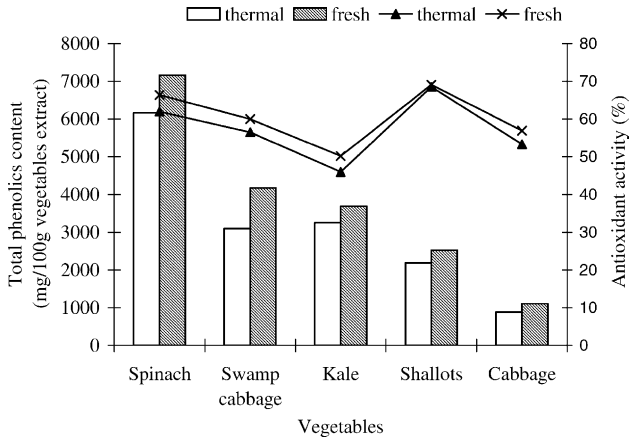

Fig. 5. Comparison of antioxidant activity and total phenolic content in fresh and thermally treated vegetables.

tivity and phenolic content was found in the study by Kähkönen et al. (1999) on some plant extracts containing phenolic compounds.

In this study, the findings do not show any relationship between antioxidant activity and total phenolic contents (Fig. 5). For example, cabbage had the lowest total phenolic content whereas its antioxidant activity was a bit higher than kale that had a higher total phenolic content. Spinach had much higher total phenolic content than other vegetables although its antioxidant activity was lower than was shallots (Fig. 5). There is a wide degree of variation between different phenolic compounds in their effectiveness as antioxidant (Robards, Prenzler, Tucker, Swatsitang, & Glover, 1999). Flavonoids generally have more hydroxyl groups if compared to ferulic acids. Besides, orthosubstitution with electron-donating alkyl or methoxy groups of flavonoid, increases the stability of the free radical and hence its antioxidant potential (Rice-Evans, Miller, Bolwell, Bramley, & Pridham, 1995). Therefore, shallots that have high flavonoid content generally have higher antioxidant activity than the other studied vegetables.

The findings of this study indicate that each type of vegetable had a different antioxidant activity, contributed by different antioxidant components, such as α-tocopherol, β-carotene, vitamin C, selenium or phenolic compounds. The high antioxidant activity of shallots might be due to its flavonoid contents; for spinach and swamp cabbage the high α-tocopherol, β-carotene and ferulic acid corresponded with the high antioxidant activity.

Acknowledgements

The authors would like to acknowledge the assistance of laboratory staff from the Department of Nutrition and Health Sciences throughout the research project. They also wish to extend their thanks to Universiti Putra Malaysia for the use of the laboratory facilities.

References

Amin, I., & Tan, S. H. (2002). Antioxidant activity of selected seaweeds. *Malaysian Journal of Nutrition, 8*, 167–177.

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

Gazzani, G., Papetti, A., Massolini, G., & Daglia, M. (1998). Anti- and prooxidant activity of water soluble components of some common diet vegetables and the effect of thermal treatment. *Journal of Agricultural and Food Chemistry, 46*, 4118–4122.

Ho, C.-T. (1992). Phenolic compounds in food: An overview. In M.-T. Huang, C.-T. Ho, & C. Y. Lee (Eds.), *ACS symposium series: 507. Phenolic compounds in food and their effects on health II: Antioxidants and cancer prevention* (pp. 2–7). Washington, DC: American Chemical Society.

Kähkönen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J. P., Pihlaja, K., Kujala, T. S., & Heinonen, M. (1999). Antioxidant activity of plant extracts containing phenolic compounds. *Journal Agricultural and Food Chemistry, 47*, 3954–3962.

Kurilich, A. C., Tsau, G. J., Brown, A., Howard, L., Klein, B. P., Jeffery, E. H., Kushad, M., Wallig, M. A., & Juvik, J. A. (1999). Carotene, tocopherol, and ascorbate contents in subspecies of *Brassica oleracea*. *Journal of Agricultural and Food Chemistry, 47*, 1576–1581.

Lindley, M. G. (1998). The impact of food processing on antioxidants in vegetable oils, fruits and vegetables. *Trend in Food Science and Techonology, 9*, 336–340.

Loliger, J. (1991). The use of antioxidants in foods. In O. I. Aruoma & B. Halliwell (Eds.), *Free radicals and food additives* (pp. 121–150). London: Taylor Francis.

Meyer, A. S., Heinonen, M., & Frankel, E. N. (1998). Antioxidant interactions of catechin, cyanidin, cafeic acid, quercetin, and ellagic acid on human LDL oxidation. *Food Chemistry, 61*, 71–75.

Micozzi, M. S., Beecher, G. R., Taylor, P. R., & Khachik, F. (1990). Carotenoid analysis of raw and cooked foods associated with a lower risk for cancer. *Journal of the National Cancer Institute, 82*, 282–285.

Namiki, M. (1990). Antioxidant/antimutagens in food. *CRC Critical Review Food Science and Nutrition, 29*, 273–300.

Papas, A. M. (1999). Diet and antioxidant status. In A. M. Papas (Ed.), *Antioxidant status, diet, nutrition, and health*. Boca Raton, London, New York, Washington, DC: CRC Press.

Puwastein, P., Burlingame, B., Raroengwichit, M., & Sungpuag, P. (2000). *ASEAN food composition tables. ASEAN network of food data system (ASEANFOODS)*. Thailand: Institute of Nutrition, Mahidol University.

Rice-Evans, C., Miller, N. J., Bolwell, P. G., Bramley, P. M., & Pridham, J. B. (1995). The relative antioxidant activity of plant-derived polyphenolic flavonoids. *Free Radical Research, 22*, 375–383.

Robards, K., Prenzler, P. D., Tucker, G., Swatsitang, P., & Glover, W. (1999). Phenolic compounds and their role in oxidative processes in fruits. *Food Chemistry, 66*, 401–436.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Tee, E. S., Lim, C. L., Chong, Y. H., & Khor, S. C. (1996). A study of the biological utilization of carotenoids of carrot and swap cabbage in rats. *Food Chemistry, 56*, 21–32.

Tsuda, T., Watanabe, M., Ohshima, K., Yamamoto, A., Kawakishi, S., & Osawa, T. (1994). Antioxidative components isolated from

the seed of tamarind (*Tamarindus indica* L.). *Journal of Agricultural and Food Chemistry, 42*, 2671–2674.

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.

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.