

Antioxidant Capacity and Phenolic Content of Selected Commercially Available Cruciferous Vegetables

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ABSTRACT

Antioxidant activity, free radical scavenging activity and phenolic content of red cabbage (*Brassica oleracea* var. *capitata rubra*), Chinese cabbage (*Brassica rapa pekinensis* var. *cylindrica*), green cabbage (*Brassica oleracea* var. *capitata*), mustard cabbage (*Brassica juncea* var. *rugosa*) and Chinese white cabbage (*Brassica rapa* var. *chinensis*), grown in Malaysia, were evaluated. Red cabbage had the highest antioxidant activity and phenolic content compared to the other cruciferous vegetables studied ($p < 0.05$). The contributions of all cruciferous vegetables to the antioxidant activity was $>79\%$. The radical scavenging activity was in the order of Chinese white cabbage $>$ red cabbage $>$ mustard cabbage $>$ Chinese cabbage $>$ green cabbage. There was a significant difference ($p < 0.05$) in the means of scavenging activity observed between cabbage, Chinese cabbage and Chinese mustard. Phenolic content was significantly different ($p < 0.05$) among all the cruciferous vegetables studied, and was in the order of red cabbage $>$ Chinese white cabbage $>$ green cabbage $>$ Chinese cabbage $>$ mustard cabbage. The study indicated that red cabbage possessed the highest antioxidant capacity and phenolic compounds concentration among all the cruciferous vegetables studied.

INTRODUCTION

Antioxidants can be broadly defined as any substance that, when present at low concentration compared to those of an oxidisable substrate, significantly prevents or delays any oxidation of that substrate (Halliwell & Gutteridge, 1990). In other words, antioxidants are compounds that inhibit or delay the oxidation of other molecules by inhibiting the initiation or propagation of oxidising chain reactions. Studies have shown that free radicals present in the human organism cause oxidative damage to different molecules, such as lipids, proteins and nucleic acids and

thus are involved in the initiation phase of some degenerative diseases. With that, the role of antioxidants has drawn much attention as a candidate to combat certain diseases and prevent the aging process (Slater, 1991).

Consumption of vegetables and fruits may help to reduce the risk of many age-related degenerative diseases. Green leafy vegetables, fruits, wheat germ, nuts and vegetable oils are excellent sources of antioxidant components (Weisburger, 1991; Lindley, 1998). Generally, it is assumed that the responsible dietary constituents contributing to the protective effects are the antioxidant vitamins such as

vitamins A, C and E. In addition, vegetables contain a group of natural antioxidants that possess not only high antioxidant activity but also good antioxidant quality.

Besides antioxidant vitamins, vegetables also contain phenolic antioxidants that may account, in part, for specific protective effects. Record, Dreosti & McInerney (2001), on examining the effects of the consumption of a high-fruit and vegetable diet, showed a significant increase in plasma antioxidants (ascorbic acid, alpha- and beta-carotene, lutein and zeaxanthin).

Cruciferous vegetables have relatively abundant sources of antioxidant substances with potential anticarcinogenic activity (Kurilich *et al.*, 1999). These vegetables are known to be rich in antioxidant substances such as ascorbic acid, β -carotene and carotenoids (Yamaguchi, 1983). Both epidemiological studies and experimental research indicated that regular intake of cruciferous vegetables may reduce risk for chronic diseases (Johnston, Taylor & Hampl, 2000; Witte *et al.*, 1996). On the other hand, low consumption of these vegetables may reduce serum antioxidant capacity (Cao *et al.*, 1998).

Vegetables commonly consumed by the population of Malaysia include cabbage, Chinese mustard, onions, long beans, swamp cabbage and spinach. Food balance sheet data for Malaysia showed that per capita of vegetables supply per year was 37.60 kg (FAO, 2005). Actual vegetable consumption data are lacking. Mubarik (1996) reported that in 1991 Malaysians consumed 4.65 kg cabbage, 2.90 kg swamp cabbage, 2.45 kg spinach and 0.77 kg kale. Recent studies showed that these vegetables have high antioxidant capacity (Amin, Zamaliah & Chin, 2004; Amin & Lee, 2005; Amin, Norazaidah & Emmy Hainida, 2006).

In this study, five types of cruciferous vegetables from *Brassica oleracea*, *Brassica juncea* and *Brassica campestris*

groups were selected. These vegetables are popular in Malaysia; and easily available in the local markets. The study aimed to investigate the antioxidant capacity and phenolic content of selected fresh cruciferous vegetables.

MATERIALS AND METHODS

Plant materials

Samples of vegetables, namely red cabbage (*Brassica oleracea* var. *capitata rubra*), Chinese cabbage (non-heading cabbage) (*Brassica rapa pekinensis* var. *cylindrica*), green cabbage (*Brassica oleracea* var. *capitata*), mustard cabbage (*Brassica juncea* var. *rugosa*) and Chinese white cabbage (*Brassica rapa* var. *chinensis*) were purchased from a wet market at Sri Serdang, Selangor, Malaysia.

Chemicals

Linoleic acid, α -tocopherol, β -carotene, 2, 2-diphenyl-1-picrylhydrazyl (DPPH), gallic acid and Tween 20, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu reagent and methanol were purchased from Merck (Darmstadt, Germany). Chloroform was from BDH Chemicals (Poole, UK) and sodium bicarbonate was from May & Baker Chemicals (Dagenham, England).

Preparation of vegetable extracts

One kilogram of each vegetable was weighed and manually chopped into small pieces using a sharp knife. Each sample was then lyophilised in a freeze-dryer (Virtis Route, Gardiner, New York). The lyophilised vegetables were ground into small particles using a dry blender (MX-291N National, Selangor, Malaysia) and kept at -20°C for further use. Preparation of vegetable extract was carried out by mixing 0.3 g of lyophilised vegetables with

30 ml of 70% methanol in a conical flask. The mixture was shaken at 200 rpm for 1 hr at room temperature using an orbital shaker (Heidolph Unimax 1010, Schwabach, Germany). The extracts were then filtered through a filter paper (Whatman No. 4) to obtain a clear solution.

Determination of antioxidant activity

β -Carotene oxidation model system

The antioxidant activity of the vegetable extracts, standard (α -tocopherol) and control (70% methanol) were determined according to the β -carotene bleaching assay following the modified procedure, initially described by Velioglu *et al.* (1998). In this assay, non-oxygenated deionised water was used and the absorbance was read at 15 min intervals instead of 10 min intervals to record the bleaching rate of β -carotene. One milliliter of β -carotene (0.2 mg/ml chloroform) was pipetted into a round-bottom flask containing 0.02 ml of linoleic acid and 0.2 ml of Tween 20. Thereafter, it was evaporated to dryness under vacuum at 40°C for 10 min using a rotary evaporator (Laborata 4000, Heidolph Instruments, Japan). Then, 100 ml of deionised water was added into the mixture and shaken vigorously until a uniform emulsion was obtained. Five milliliters aliquot of the emulsion was transferred to each test tube containing 0.2 ml of vegetables extracts or α -tocopherol. The mixture was then vortexed for 1 min and incubated in a water bath (Protech 830-S1, Kuala Lumpur, Malaysia) at 45 °C for 2 hr. The absorbance was monitored using a UV-1601 UV-Visible spectrophotometer (Shimadzu, New South Wales, Australia) at 470 nm. Absorbance was recorded every 15 min intervals against a blank which consisted of the same emulsion but without β -carotene. All samples were determined in duplicate.

The antioxidant activity (AA) was calculated according to the following equation.

$$AA = [1 - (A_o - A_t) / (A_o^o - A_o^t)] \times 100$$

where, A_o and A_o^o = absorbance values measured at zero time of incubation for test sample and control respectively, while A_t and A_o^t = absorbance measured in the test sample and control, respectively, after incubation for 120 min.

2, 2-diphenyl-1-picrylhydrazyl radical (DPPH) inhibition system

The free radical scavenging activity of the vegetable extracts and the ascorbic acid (standard) were determined according to the DPPH free radical scavenging assay described by Tang *et al.* (2002). 200 μ l of vegetable extract at a concentration of 10 mg/ml or standard was added to 1 ml of 0.2 mM DPPH in methanol. The mixture was then shaken vigorously and kept to stand in the dark room for 30 min at room temperature. The absorbance was read at 517 nm with deionised as blank. The readings were compared with the controls which contained 200 μ l of 70% methanol and 1 ml DPPH served as the control. All samples were measured in duplicate. The radical scavenging activity (%) was calculated according to the equation as follows:

Scavenging activity (%) =

$$1 - \left[\frac{\text{absorbance of sample at 517 nm}}{\text{absorbance of control at 517 nm}} \right] \times 100$$

Determination of total phenolic content

The total phenolic content of the vegetable extracts were determined according to the Folin-Ciocalteu assay following the procedure described by Velioglu *et al.* (1998). The lyophilised vegetables (0.1 mg) were extracted with 20 ml of 70% methanol by placing the mixture on the orbital shaker (Heidolph Instruments, state, Germany) at 200 rpm for 2 hr at room temperature (28°C). An aliquot (200 μ l) of clear extract was mixed with 1.5 ml Folin-Ciocalteu reagent (diluted 10-fold

with deionised water) and then left to stand at room temperature. Following 5 min, 1.5 ml of sodium bicarbonate (0.6 M) was added to the reaction and then left to stand at room temperature. Following 90 min, the absorbance was read at 725 nm. Gallic acid was used as the standard for estimating the total phenolic content of the vegetable extracts. A standard calibration curve of 0.001 – 0.007 mg/ml gallic acid in 70% methanol was plotted. The total phenolic content of the vegetable extracts was expressed as gallic acid equivalents (GAE) in mg per 100 g fresh weight.

RESULTS AND DISCUSSION

Solvent extraction is frequently used for isolation of antioxidants. Antioxidant

activity of extracts is strongly dependent on the solvent, due to different antioxidant potentials of compounds with different polarity (Marinova & Yanishlieva, 1997; Soong & Barlow, 2004). The methanol-water systems were used as extraction solutions in the present study as they are the most universal and widely employed solvents for antioxidant extractions. Moreover, antioxidant activity was found to be higher in methanol extracts compared to ethanol and other organic solvents (Sun & Ho, 2005).

The bleaching rate of β -carotene in the presence of different types of methanolic vegetable extracts is shown in Figure 1. The control sample that contained no antioxidant component had the highest bleaching rate, while α -tocopherol (vita-

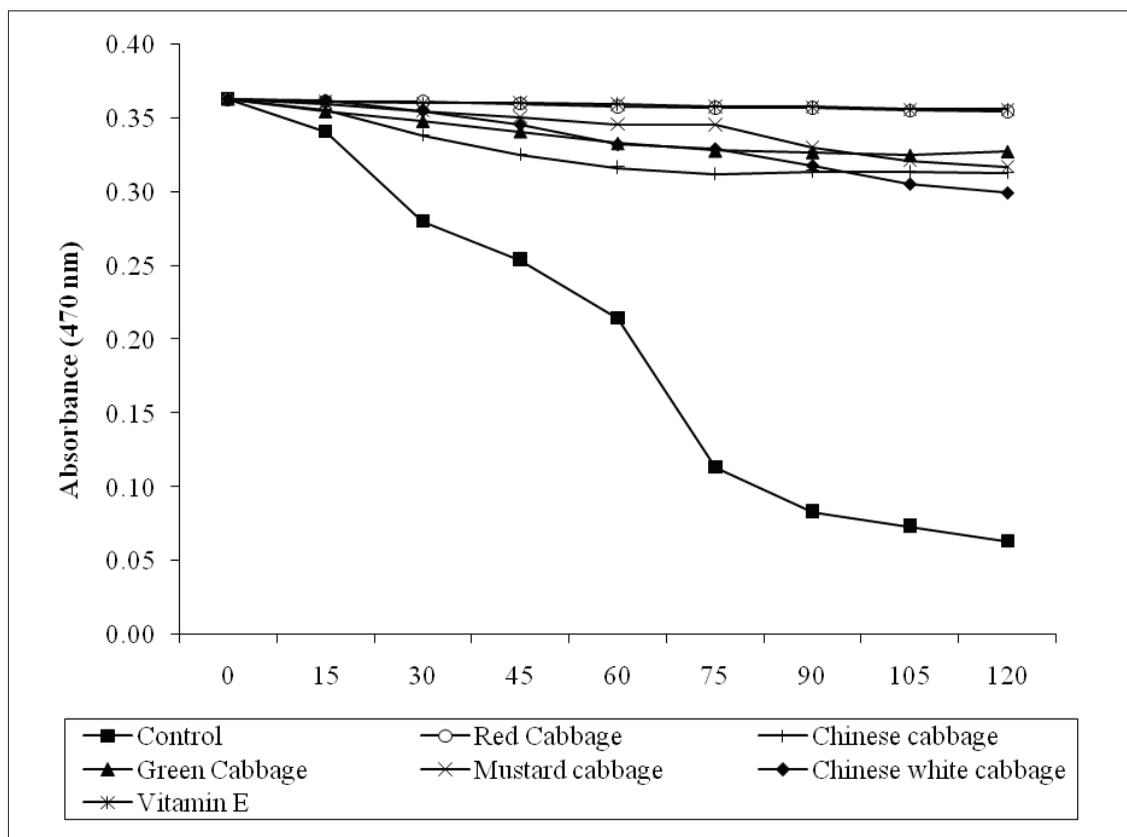


Figure 1. Antioxidant activity of fresh vegetables methanolic extract compared with vitamin E at 10 mg/ml using a β -carotene bleaching assay. Coefficients of variance (CV) are less than 6%

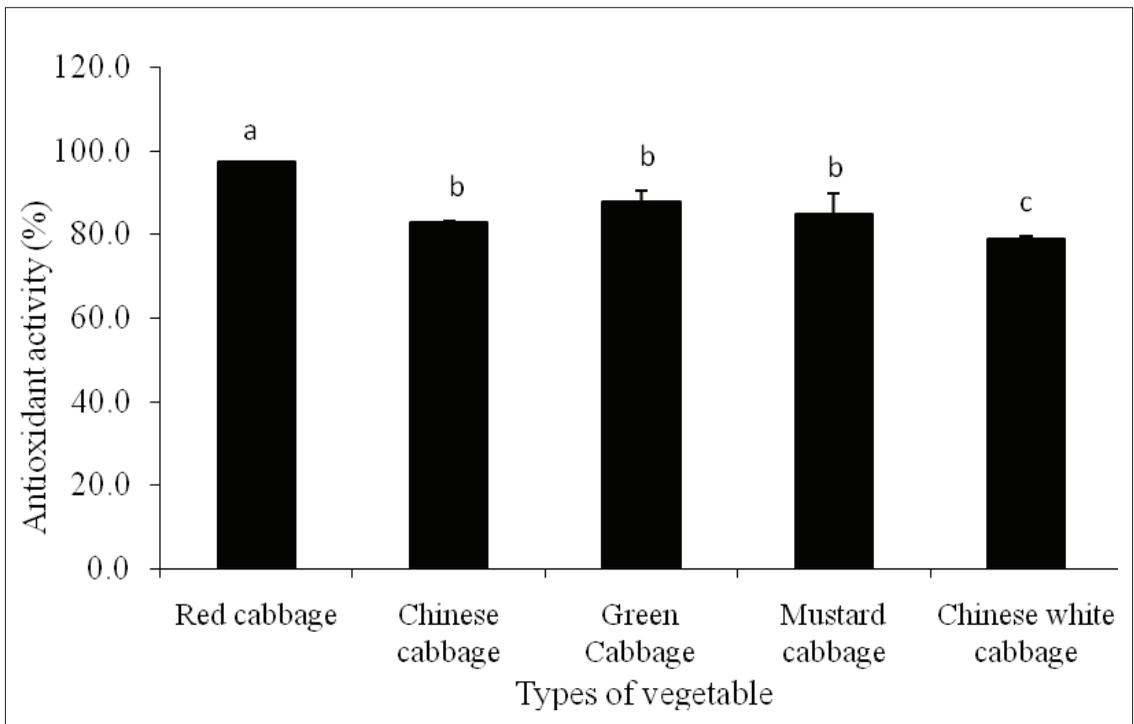


Figure 2. Antioxidant activity of different types of fresh vegetables. Values are expressed as mean standard deviation of three replicate determinations. Different letters indicate significant difference at $p < 0.05$. Antioxidant activity was measured using a β -carotene bleaching assay. Values indicate that the coefficient of variation was less than 10%

min E) inhibited the oxidation of β -carotene efficiently and had the highest antioxidant activity compared to all the vegetable extracts.

Among the vegetable extracts (Fig. 2), red cabbage had the highest antioxidant activity (97%), followed by green cabbage (88%), mustard cabbage (85%), Chinese cabbage (83%), and Chinese white cabbage (79%). There was a significant difference ($p < 0.05$) in the mean antioxidant activity of red cabbage with other methanolic extracts. However, no significant difference was found between red cabbage and vitamin E. There was also a significant difference ($p < 0.05$) in the mean of antioxidant activity between Chinese white cabbage and green cabbage. However, no significant difference was found in the means of antioxidant activity among Chinese white cabbage, Chinese

cabbage and mustard cabbage. In addition, the antioxidant activity of green cabbage, mustard cabbage and Chinese cabbage also showed no significant difference among them.

The result with red cabbage having the highest antioxidant activity compared to the other green and white cultivars was in agreement with Furuta, Nishiba & Suda (1997). This may suggest the presence of anthocyanidin components. Wang, Cao & Prior (1997) and Tsuda *et al.* (1994) reported that anthocyanin pigments contained potent antioxidant properties. Furthermore, methanolic extracts of anthocyanin-rich plant materials showed strong antioxidative activities in a β -carotene bleaching method as reported by Velioglu *et al.* (1998). Wills *et al.* (1984) studied the nutrient composition of Chinese vegetables and found that the carotenes present in

Chinese cabbage, mustard cabbage and Chinese white cabbage are α -, β -carotene and cryptoxanthin. The order of antioxidant activity of the vegetables obtained in our study was similar to the study of Wills *et al.* (1984).

According to Tee *et al.* (1997), mustard cabbage had the highest vitamin C content, followed by Chinese white cabbage, green cabbage and Chinese cabbage. In addition, Wills *et al.* (1984) found the vitamin C content to be highest in mustard cabbage followed by Chinese white cabbage and Chinese cabbage.

The presence of vitamin C might be responsible for the high antioxidant activi-

ty of mustard cabbage and Chinese white cabbage. The antioxidant activity of all vegetables extracted with methanol might be due to the differences in solubility and extractability of the antioxidant compounds contained in each type of vegetable extracts.

Chinese white cabbage had an exceptionally high radical scavenging activity of 97%, which was even higher than the standard. Red cabbage, mustard cabbage and Chinese cabbage possessed a high scavenging activity too, which were 96%, 89% and 84%, respectively. However, green cabbage had the lowest radical scavenging activity among those vegetables extracted

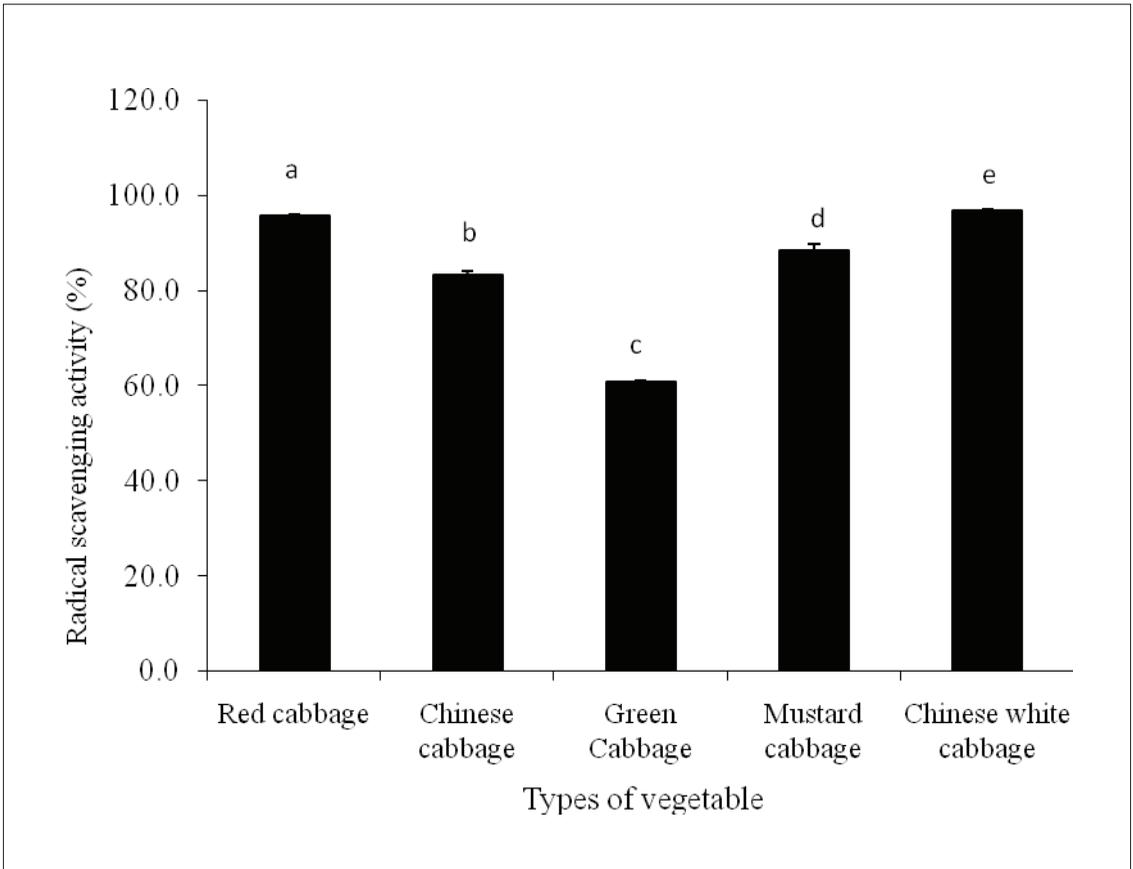


Figure 3. Scavenging activity of different types of fresh vegetables. Values are expressed as mean standard deviation of three replicate determinations. Different letters indicate significant difference at $p < 0.05$. Free radical scavenging activity was measured using a DPPH radical scavenging assay. Values indicate that the coefficient of variation was less than 13%

with methanol (Fig. 3). The free radical scavenging activity of Chinese white cabbage > red cabbage > mustard cabbage > Chinese cabbage > green cabbage. Amin & Lee (2005) had reported that the free radical scavenging activity of these cruciferous vegetable water extracts was in the order of red cabbage > Chinese cabbage > green cabbage > mustard cabbage > Chinese white cabbage.

One-way ANOVA showed a significant difference ($p < 0.05$) in the means of radical scavenging activity between vegetable extracts and the standard. The means of radical scavenging activity was also significantly different ($p < 0.05$) between green cabbage, Chinese cabbage and both Chinese mustards. In the study by Gil *et al.* (2000), anthocyanins with the phenolic compound cyanidin 3-glucoside is an effective radical scavenger by having four free phenolic hydroxyls. As expected, red cabbage had high radical scavenging activity. However, the difference between the radical scavenging activities of Chinese white cabbage and red cabbage was less than 1%.

Vitamin C was reported to have the highest antiradical efficiency among the phenolic components (gallic acid, tannic acid, caffeic acid, quercetin, BHA, rutin, ferrulic acid, α -tocopherol and resveratrol) when using methanol as a solvent (Sánchez-Moreno, Larrauri & Saura-Calixto, 1998). It is assumed that the high content of ascorbic acid in Chinese white cabbage and mustard cabbage in this study might have accounted for their higher radical scavenging activities than the levels of Chinese cabbage and green cabbage in methanol extraction.

As Chinese cabbage and green cabbage also had low ascorbic acid content compared to mustard cabbage (Tee *et al.*, 1997), this might explain why the results of this study showed that Chinese cabbage and green cabbage had lower radical scavenging activity than that of mustard cabbage. However, there was no significant

difference in antioxidant activity between them (Fig. 2). The difference in both results may be due to the fact that the antioxidant activity measured with b-carotene bleaching assay was calculated based on the presence of any compound with antioxidative activity while in DPPH free radical scavenging assay, radical scavenging activity was calculated based on the presence of compounds that was able to donate electrons or scavenge DPPH radicals (Lai, Chou & Chao, 2001).

Figure 4 shows the phenolic content of 5 types of vegetables extracted with methanol. Red cabbage had the highest phenolic content of 439 mg GAE among the vegetable extracts. Chinese white cabbage had a total phenolic content of 96 mg GAE, green cabbage (85 mg GAE), Chinese cabbage (67 mg GAE) and mustard cabbage had the least phenolic content among all the fresh vegetable extracts (46 mg GAE). Statistical analysis showed that there was a significant difference ($p < 0.05$) in the means of total phenolic content among the vegetables.

Nearly the same sequence was observed in water extraction by Amin & Lee (2005), where phenolic content of red cabbage > Chinese white cabbage > green cabbage > mustard cabbage > Chinese cabbage. Again, red cabbage had the highest phenolic content among the vegetables despite the different types of extraction medium used. As seen in antioxidant activity and free radical scavenging activity, red cabbage possessed very high antioxidant potential, most probably due to its anthocyanin content that was absent in other white and green cultivars used in this study (Furuta, Nishiba & Suda, 1997; Wang, Cao & Prior, 1997; Tsuda *et al.*, 1994).

Kähkönen *et al.* (1999) reported that among 92 types of edible and non-edible plant materials, low levels of phenolic content were found in vegetable extracts of onion, cucumber, carrots and others compared to berries, fruits, tree materials and

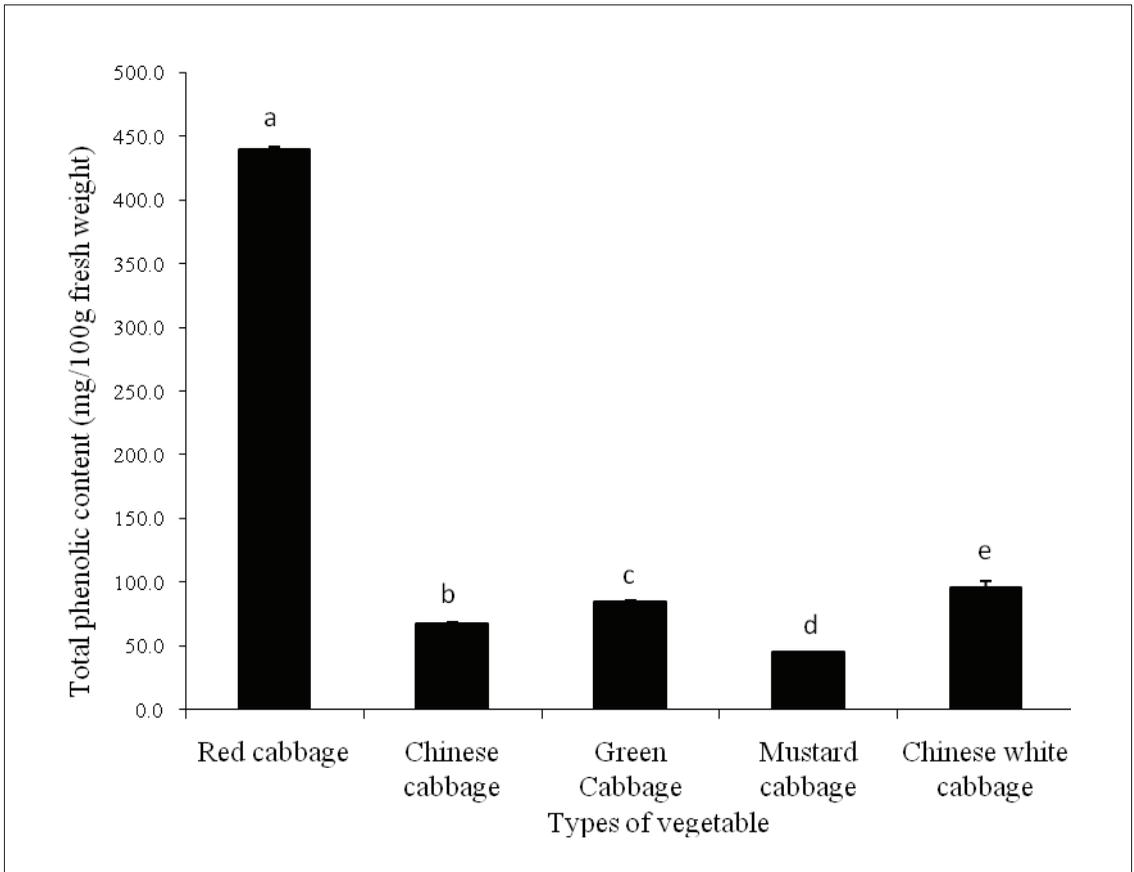


Figure 4. Total phenolic content of different types of fresh vegetables. Values are expressed as mean standard deviation of three replicate determinations. Different letters indicate significant difference at $p < 0.05$. Total phenolic content was estimated using a Folin-Ciocalteu reagent assay. Values indicate that the coefficient of variation was less than 5%

plant sprouts. Moreover, Vinson *et al.* (1998) showed that cabbage has lower phenol content than onion. As ascorbic acid is a type of phenolic antioxidant (Sánchez-Moreno, Larrauri & Saura-Calixto, 1998), low levels of ascorbic acid might result in a low phenolic content observed in Chinese cabbage compared to other vegetable extracts.

Among the cruciferous vegetables used in this study, red cabbage had a high antioxidant activity. Each vegetable studied has its own antioxidant compounds that might influence their antioxi-

dant and free radical scavenging activities, and total phenolic content.

CONCLUSION

Each vegetable studied has its own antioxidant compounds including polyphenols that might influence their antioxidant and free radical scavenging activities. Red cabbage (*Brassica oleracea* var. *capitata rubra*) had a high antioxidant capacity and total phenolic content among the cruciferous vegetables.

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