International Food Research Journal 19(1): 327-332 (2012)

Effects of drying methods on bioactive compounds of vegetables and correlation between bioactive compounds and their antioxidants

*Hung, P. V. and Duy, T. L.

School of Biotechnology, International University, Vietnam National University in Ho Chi Minh City, Quarter 6, Linh Trung Ward, Thu Duc District, Ho Chi Minh City, Vietnam

Abstract: Freeze- and heat-drying methods were applied to dry fresh vegetables (carrot, taro, tomato, red beetroot and eggplant) grown in Vietnam and then the total phenolic and flavonoid compounds extracted by alcoholic and alkaline-hydrolysis methods were evaluated to determine the effects of the drying methods on the bioactive compounds of the vegetables. Furthermore, the correlations between the content of bioactive compounds and their antioxidant capacity were also investigated in this study. The results show that phenolic and flavonoid compounds were mainly located in free form in the vegetables which was easily extracted by alcoholic solvent. A high temperature in the heat-drying method in sample preparation significantly reduced total free and bound phenolics, total free and bound flavonoids and their antioxidant capacity. The antioxidant capacity of the extracts highly correlated with free phenolic compounds (r^2 = 0.8936) and free flavonoid compounds (r^2 = 0.6682). In contrast, the antioxidant capacity of the extract did not correlate with the bound phenolic and flavonoid compounds (r^2 = 0.0124 and r^2 = 0.0854, respectively).

Keywords: Phenolics, flavonoids, antioxidant, fruits and vegetables

Introduction

Fruits and vegetables are rich in phenolic metabolites including tocopherols, flavonoids, phenolic acids, alkaloids, chlorophyll derivatives, or carotenoids (Hudson, 1990; Hall and Cuppett, 1997), that possess high antioxidant capacity and have significantly health benefits (McDermott, 2000). The beneficial effects derived from diets rich in fruits and vegetables is to protect against the risks for chronic angiogenic diseases, such as cardiovascular diseases, arthritis, chronic inflammation and cancers (Middleton *et al*., 2000; Saleem *et al*., 2002; Prior, 2003; Zhang *et al*., 2005; Chen *et al*., 2005). The phenolic metabolites in fruits and vegetables varies depending on the plant origins (Robards and Antolovich, 1997). Therefore, the extracts from different kinds of fruits and vegetables exhibited the different antioxidant capacity. The majority of antioxidant activity of fruits and vegetables may be derived from phenolic compounds. However, Burton and Ingold (1981) have shown that α-tocopherol is one of the most active *in vitro* chain-breaking antioxidants. In addition, carotenoids also have protective functions against oxidative damage (Krinsky, 1989).

Among the known fruits and vegetables, deep-colored fruits and vegetables have been reported to be good sources of phenolics, including flavonoids, anthocyanins and carotenoids and recognized as more healthy to human body, especially in the oriental countries (Qian *et al*., 2004; Sass-Kiss *et al*., 2005; Cieslik *et al*., 2006; Lin and Tang, 2007). Several deep-colored vegetables such as tomato (*Lycopersicon esculentum* Mill), carrot (*Daucus carrota* L.), eggplant (*Solanum melongena*), red beetroot (*Beta vulgaris*) and taro (*Colocasia esculenta*) have been reported to contain large amounts of bioactive compounds and have strong antioxidant capacity (Vinson *et al*., 1998). The antioxidant potential of tomato is derived from lycopene, ascorbic acid, phenolics, flavonoids and vitamin E, in which lycopene constitutes more than 60% of the carotenoids present (Roldan-Gutierrez and Luque de Castro, 2007). Lycopene is one of the major carotenoids in the diet of North American and European and the most important source of lycopene is tomato and its processed food products (Roldan-Gutierrez and Luque de Castro, 2007). The bioactive compounds in the eggplant fruit include phenolics, flavonoids, nasunin, ascorbic acid and vitamin A, which are antioxidants (Vinson *et al*., 1998) and possess high capacity in scavenging of superoxide free radicals and inhibition of hydroxyl radical generation by chelating ferrous iron (Kaneyuki *et al*., 1999; Noda *et al*., 2000). The antioxidant properties of carrot are derived from anthocyanins, carotenoids and phenolics, which are higher in purple carrot rather than in other coloured carrot varieties (orange, yellow and white) (Alasalvar *et al*., 2005). Betalains and anthocyanins are mutually exclusive in their natural occurrence, but phenolics and flavonoids have also been found in different beetroot materials (Stafford, 1994; Wende *et al*., 1999; Kujala *et al*., 2000), contributing to the strong antioxidant activity of beetroot feel extract (Kahkonen *et al*., 1999). Taro

*Corresponding author.
Email: *hungphambk@yahoo.com*
Tel: +84-83-724-2181/3344

© All Rights Reserved

also contains a range of phenolic compounds which includes gallic acid, chlorogenic acid, (+)-catechin, (−)-epicatechin and (−)-epigallocatechin and the possible presence of proanthocyanidins and flavonols contributing to the antioxidant activity of taro extract (Agbor-Egbe and Rickard, 1990). Along with the consumption of fresh vegetables, the dried vegetable including dried powders of vegetables have been used to produce the deep-colored foods such as tomato ketchup, carrot powder, cakes, functional foods, etc. Drying methods play an important role in production of the dried vegetables and the bioactive compounds and their antioxidant capacity might be lost during drying process.

The objective of this study is to investigate the contribution of total free and bound phenolics and flavonoids to the antioxidant capacity of extracts of selected deep-coloured vegetables grown in Vietnam including tomato, eggplant, carrot, beetroot and taro. In addition, effects of drying methods, heat- and freeze-drying methods, on total contents of total free and bound phenolics and flavonoids and their antioxidant capacity of the vegetable extracts are also investigated.

Materials and Methods

Materials

Selected deep-coloured vegetables including carrot (*Daucus carrota* L.), taro (*Colocasia esculenta*), tomato (*Lycopersicon esculentum* Mill.), red beetroot (*Beta vulgaris*) and eggplant (*Solanum melongena*) used in this study were grown at the southern region of Vietnam and are commercially popular products at local super markets. All collected vegetables were fresh and matured. All chemicals including Folin-Ciocalteu's phenol reagent, 2,2-diphenyl-1-picyrylhydrazyl (DPPH), galic acid, rutin and other solvents were purchased from Sigma-Aldrich Chemical Company (Singapore).

Sample preparation

All vegetables were washed and edible parts were used. Fleshes of carrot, taro and red beetroot were cut into a cubic of 1cm^3. Tomato seeds were removed before cutting and eggplant peels were used instead of flesh. All pieces were divided into 2 groups. The group 1 was dried in an oven at 55^{o}C overnight, while group 2 was freeze-dried overnight. After drying, all pieces were milled into flour and stored in desiccators for later use.

Extraction of phenolic compounds

Free phenolic compounds in vegetable powders were extracted according to the method of Adom and Liu (2002). The powdered vegetable (0.5 g) was extracted with 10 ml of 80% chilled ethanol for 10 min with vortex mix. The suspension were then centrifuged at 1,500 × g for 10 min then the supernatant was collected. The extraction was repeated triplicate and the combined supernatant was then evaporated at 45^{o}C and then reconstituted with methanol to a final volume of 20 ml. The free phenolic acids were then stored at -4 ^{o}C in a refrigerator until later use.

The residue from the free phenolic extraction was directly hydrolyzed with 20 ml of 2N sodium hydroxide for 24 h with stirring at room temperature. The hydrolyzed solution was acidified to pH 2 with HCl 6N. This solution was then extracted 6 times with diethyl ether-ethyl acetate (1:1). The ether/ethyl acetate extracts were evaporated to dryness and the bound phenolic acids were dissolved in 20 ml of methanol and stored at -4^{o}C until later use. All extractions were performed in duplicate.

Determination of total phenolic contents

Contents of free and bound phenolics in vegetables were determined using the Folin-Ciocalteu's colorimetric method as previously reported by Hung and Morita (2008). Extracted solution (0.5 ml) was put into a test tube and the Folin-Ciocalteu's phenolic reagent (0.5 ml) was added. The content was vortex mixed and added with 1 ml of saturated sodium carbonate solution, followed by adjusting the volume to 10 ml with distilled water. The mixtures in the tubes were thoroughly mixed by vortexing. Tubes were allowed to stand at ambient temperature for 10 min until the characteristic blue color developed. The control was prepared in same way but the extracted solution was replaced by methanol (0.5 ml). Absorbance of the clear supernatants was measured at 725 nm using a spectrophotometer (UVD-2960, Labomed, Inc.). Galic acid was used as a standard and total phenolic content were calculated and expressed as µg galic acid equivalent (GAE) per g sample. All analyses were performed in triplicate.

Determination of total flavonoid contents

Flavonoid contents of free and bound phenolics in vegetables were determined using the aluminum chloride colorimetric method of Chang *et al*. (2002). An appropriate dilution of extract (0.5 ml) was mixed with 1.5 ml of 95% ethanol, followed by 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was recorded at 415 nm. Rutin was used as a standard and total flavonoid contents

were calculated and expressed as µg rutin equivalent (RE) per g sample. All analyses were performed in duplicate.

DPPH scavenging capacity

DPPH free radical scavenging capacities of vegetable extracts were determined by the reduction of reaction color between DPPH solution and sample extracts according to the method of Huang *et al*. (2005) with minor modifications. The concentration of DPPH solution used was 0.1 mM. Mixture of DPPH solution (3.9 ml) and extracted sample (0.1 ml) was kept in the dark at the ambient temperature. Absorbance of mixtures was recorded at 525 nm for exactly 30 min. The control was made from 3.9 ml of DPPH solution and 0.1 ml of methanol and then measured at t = 0.

The DPPH scavenging was calculated according to the following equation:

$$\%\ DPPH\ scavenging = \frac{Abs(t=0) - Abs(t=30)}{Abs(t=0)} \times 100$$

Where: Abs(t=0) is absorbance of DPPH radical and methanol at t = 0 and Abs(t=30) is absorbance of DPPH radical and extracts at t = 30.

Statistical analysis

All analyses were performed in triplicate. Analysis of variance (ANOVA) was performed using Duncan's multiple-range test to compare treatment means at $P < 0.05$ using SPSS software version 16 (SPSS Inc., USA).

Results and Discussion

Total Phenolic Content (TPC)

Total phenolic contents of free and bound phenolic extracts from the vegetables by freeze- and heat-drying methods are shown in Table 1. Total phenolics contents of free phenolic extracts of each kind of vegetables in both drying methods were significantly higher than those of the bound phenolic extracts. These results indicate that the phenolic compounds in vegetables existed primarily in free form rather than in bound form and solvent extraction methods were simple and effective methods for phenolic recovery. By freeze-drying, free phenolic contents in vegetables were increased in order: carrot < tomato < taro < beetroot < eggplant, in which eggplant contained the highest amount of free phenolic compound (454.5 µg GAE/g db sample) and carrot had the lowest (36.6 µg GAE/g db sample). Bound phenolic contents of the selected vegetables ranged from 3.3 to 19.2 µg GAE/g db sample. The highest level of bound phenolics was also found in eggplant (19.2 µg GAE/g db sample), whereas the lowest bound phenolics was in taro (3.3 µg GAE/g db sample). Lin and Tang (2007) reported that the total phenolic and flavonoid contents in fruits and vegetables varied considerably and direct determination of each fruit and vegetables is a practical method to screen phenolic-rich fruits and vegetables but not only colors. Therefore, the results in this study indicate that eggplant and beetroot are two vegetables which are good sources of phenolics.

Table 1. Total phenolic compounds of several vegetables with different drying methods

Samples	Total phenolic compounds (µg GAE/ g sample, db)			
	Freeze-Drying		Heat-Drying	
	Alcoholic extract	Alkaline extract	Alcoholic extract	Alkaline extract
Carrot	36.6 ± 0.5a	6.7 ± 0.9b	33.1 ± 0.5c	4.6 ± 0.5a
Taro	93.5 ± 0.9c	3.3 ± 0.7a	8.6 ± 0.5a	5.8 ± 0.7a
Tomato	61.2 ± 0.7b	11.1 ± 0.7c	21.6 ± 1.0b	19.4 ± 0.5b
Beetroot	229.2 ± 1.6d	17.8 ± 0.7d	31.4 ± 0.8c	24.3 ± 1.4c
Eggplant	454.5 ± 0.5e	19.2 ± 0.7d	49.2 ± 0.7d	26.1 ± 0.2c

[a] All values are means of two extractions.
[b] The same letter in the same column is not significant different ($p<0.05$).

Table 1 also shows the loss of free phenolic compounds in the vegetable during drying. Total phenolic contents in free phenolic extracts found in freeze-drying vegetables were significantly higher than those in heat-drying vegetables. Total free phenolics of freeze- and heat-drying eggplant, for instance, were 454.5 and 49.2 µg GAE/g db sample, respectively. In beetroot extract, it was nearly 13 times different (229.2 and 17.8 µg GAE/g db sample) in comparison between freeze- and heat-drying vegetables. Although free phenolics were significantly higher in freeze-drying vegetables, their bound phenolics were slightly lower as compared with the heat-drying vegetables. Thus, the bound phenolics were not affected by drying methods because of their association with cell wall of vegetables. Drying is an important method of food preservation, which provides longer shelf-life, lighter weight for transportation and smaller space for storage. However, this study indicates that drying vegetables by heat which has been popularly used in food processing reduces significantly amounts of free phenolic compounds resulting in lowering the health benefit of vegetables.

Total flavonoid content (TFC)

Total flavonoid contents of free and bound phenolic extracts from freeze- and heat-drying vegetables are listed in Table 2. In free form, flavonoid contents of carrot, taro, tomato, beetroot and eggplant dried by freeze-drying method were 11.2, 7.1, 14.7, 15.3 and 42.1 µg RE/g db sample, respectively. As a result, eggplant was found to contain the highest level of

free flavonoid (42.1 µg RE/g db sample) and taro was the lowest (7.1 µg RE/g db sample). The total flavonoid contents of free phenolic extracts were not quite different in both drying method. The significant reduction was account for beetroot flavonoid which were about 1.7 times reduction. Flavonoids, in bound form, of freeze-drying vegetables ranged from 3.57 to 5.41 µg RE/g db sample, whereas those of heat-drying vegetables ranged from 2.7 to 6.3 µg RE/g db sample. Similar to the total phenolic contents, the flavonoid content of the free phenolic extracts was higher than that of the bound phenolic extracts. The high amount of free flavonoid compared to the bound flavonoid was different from other investigations in grains such as rice, wheat, corn and oat (Adom and Liu, 2002; Hung *et al*., 2011). Oomah and Mazza (1996) reported that the quantity of flavonoid varied depending on the cultivar and environment effects. Thus, in this study, the highest level of total flavonoid contents was found in eggplant (46.4 and 41.5 in freeze- and heat-drying eggplant, respectively), while the lowest was in taro (10.7 and 10.9 in freeze- and heat-drying taro, respectively). The results indicate that eggplant feel extract is a good source of phenolic and flavonoid compounds and has more benefits to human health.

Table 2. Total flavonoid compounds of several vegetables with different drying methods

Samples	Total flavonoid compounds (µg RE/ g sample, db)			
	Freeze-Drying		Heat-Drying	
	Alcoholic extract	Alkaline extract	Alcoholic extract	Alkaline extract
Carrot	11.2 ± 0.2b	5.4 ± 0.2d	8.9 ± 0.2b	5.6 ± 0.1c
Taro	7.1 ± 0.5a	3.6 ± 0.2a	6.2 ± 0.3a	4.7 ± 0.2b
Tomato	14.7 ± 0.2c	4.9 ± 0.2c	15.1 ± 0.1c	6.3 ± 0.4d
Beetroot	15.3 ± 0.9c	4.6 ± 0.1bc	9.1 ± 0.1b	4.9 ± 0.1b
Eggplant	42.1 ± 0.2d	4.3 ± 0.2b	38.8 ± 0.1d	2.7 ± 0.2a

[a] All values are means of two extractions.
[b] The same letter in the same column is not significant different (p<0,05).

DPPH radical scavenging of free and bound phenolic compounds

Scavenging of the stable DPPH radical was widely used to evaluate antioxidant activity of phenolic compounds extracted from fruit and vegetables, cereal, grain, wine, etc. In this study, the antioxidant activities of free and bound phenolic extracts of the selected vegetables were evaluated using DPPH assay. DPPH scavengings of free and bound forms of phenolic extracts from the freeze- and heat-drying vegetables are shown in Table 3. DPPH radical scavenging of free phenolic extracts was highest in eggplant (92.3%) and the lowest in carrot (12.6%). The highest level of DPPH radical scavenging of the bound phenolic extracts was 6.9% in eggplant, whereas tomato was the lowest (1.3%). DPPH scavenging of free phenolic extracts from the heat-drying vegetables was also high even though the amounts of free phenolic compounds reduced significantly as affected by heat-drying method. DPPH radical scavenging of bound phenolic extracts was significant lower than that of free phenolic extracts of both freeze- and heat-drying vegetables. Thus, the free phenolic extracts of vegetables exhibited the significant antioxidant capacity which contributes to human health benefits.

Table 3. DPPH radical scavenging (%) of phenolic extracts of several vegetables with different drying methods

Samples	DPPH radical scavenging (%)			
	Freeze-Drying		Heat-Drying	
	Alcoholic extract	Alkaline extract	Alcoholic extract	Alkaline extract
Carrot	12.6 ± 0.3a	5.5 ± 0.3c	6.7 ± 0.3a	2.4 ± 0.1b
Taro	48.5 ± 0.1c	5.3 ± 0.1c	30.6 ± 1.3c	2.1 ± 0.3b
Tomato	26.0 ± 0.3b	1.3 ± 0.1a	24.0 ± 1.6b	1.3 ± 0.2a
Beetroot	50 ± 2.7b	4.2 ± 0.6b	46.1 ± 3.3d	5.5 ± 0.1c
Eggplant	92.3 ± 0.5d	6.9 ± 0.3d	86.9 ± 0.1e	2.3 ± 0.1b

[a] All values are means of two extractions.
[b] The same letter in the same column is not significant different (p<0.05).

Correlation between DPPH radical scavenging and TPC and TFC in vegetables

The high correlation between free phenolics and DPPH scavenging in vegetables is shown in Figure 1A with $r^2 = 0.8936$ whereas no correlation between bound phenolics and DPPH scavenging is observed with $r^2 = 0.0124$ (Figure 1B). These results indicate that antioxidant properties were strongly correlated to the concentration of free phenolics extracted from vegetables rather than bound phenolics concentration.

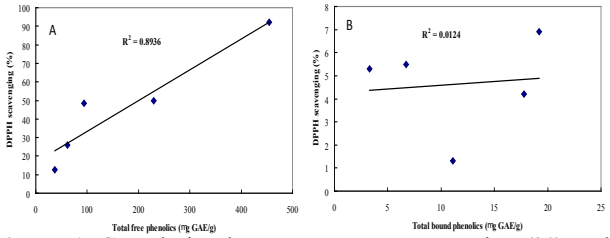

Figure 1. Correlation between DPPH scavenging (%) and total free (A) and bound (B) phenolic extracts

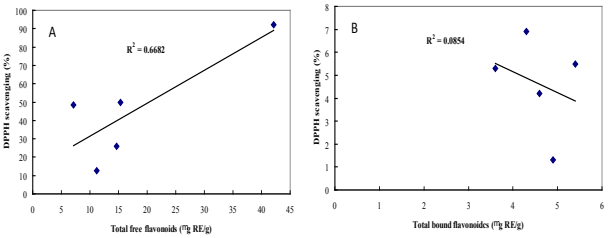

Figure 2. Correlation between DPPH scavenging (%) and total free (A) and bound (B) flavonoid extracts

As shown in Figure 2A, free flavonoid concentration was slightly correlated with DPPH scavenging (r^2 = 0.6682). However, no correlation between bound flavonoid and DPPH scavenging (r^2 = 0.0854) was also observed (Figure 2B). These results show that the free flavonoids in vegetables also contributed to the antioxidant property of the extracts.

Conclusions

The results of this study show that the total phenolic and flavonoid contents in vegetables were significantly higher by freeze-drying method than the conventional heat-drying method. The phenolic acids and flavonoids are mainly in free forms, which can easily extracted using ethanol or methanol solvents. As the result, in vegetables, the higher phenolic contents exhibited the higher antioxidant capacity and flavonoid compounds also contribute to the antioxidant properties of vegetable extracts

Acknowledgement

The authors acknowledge with thanks the financial support received under Research Grant No. 106.9902010.66 from National Foundation for Science and Technology Development, Vietnam.

References

Adom, K. K. and Liu, R. H. 2002. Antioxidant activity of grains. Journal of Agricultural and Food Chemistry 50: 6182-6187.

Agbor-Egbe, T. and Rickard, J. E. 1990. Identification of phenolic compounds in edible aroids. Journal of the Science of Food and Agriculture 52: 215-221.

Alasalvar, C., Al-Farsi, M., Quantick, P. C., Shahidi, F. and Wiktorowicz, R. 2005. Effect of chill storage and modified atmosphere packaging (MAP) on antioxidant activity, anthocyanins, carotenoids, phenolics and sensory quality of ready-to-eat shredded orange and purple carrots. Food Chemistry 89: 69-76.

Burton, G. W. and Ingold, K. U. 1981. Autoxidation of biological molecules. 1. Antioxidant activity of vitamin E and related chain-breaking phenolic antioxidants in vitro. Journal of the American Chemical Society 103: 6472-6477.

Chang, C.-C., Yang, M.-H., Wen, H.-M. and Chern, J.-C. 2002. Estimation of total Flavonoid content in propolis by two complementary colorimetric methods. Journal of Food and Drug Analysis 10: 178-182.

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

Chen, C.-C., Liu, L.-K., Hsu, J.-D., Huang, H.-P., Yang, M.-Y. and Wang, C.-J. 2005. Mulberry extract inhibits the development of atherosclerosis in cholesterol-fed rabbits. Food Chemistry 91: 601–607.

Hall, C. A. and Cuppett, S. L. 1997. Structure-activities of natural antioxidants. In Aruoma, O. I., and Cuppett, S. L. (Eds). Antioxidant Methodology *In Vivo* and *In Vitro* Concepts, pp 2-29. Champaign, IL: AOCS Press.

Hudson, B. J. F. 1990. Food Antioxidants. London: Elsevier Applied Science.

Huang, D., Ou, B. and Prior, R. L. 2005. The chemistry behind antioxidant capacity assays. Journal of Agricultural and Food Chemistry 53: 1841-1856.

Hung, P. V., Hatcher, D. W. and Barker, W. 2011. Phenolic acid composition of sprouted wheats by ultra-performance liquid chromatography (UPLC) and their antioxidant activities. Food Chemistry 126: 1896-1901.

Hung, P. V. and Morita, N. 2008. Distribution of phenolic compounds in the graded flours milled from whole buckweak grains and their antioxidant capacities. Food Chemistry 109: 325-331.

Kahkonen, M. P., Hopia, A. I., Vuorela, H. J., Rauha, J.-P., Pihlaja, K., Kujala, T. S. and Heinonen, M. 1999. Antioxidant activity of plant extracts containing phenolic compounds. Journal of Agricultural and Food Chemistry 47: 3954-3962.

Kaneyuki, T., Noda, Y., Traber, M.G., Mori, A. and Packer, L. 1999. Superoxide anion and hydroxyl radical scavenging activities of vegetable extracts measured using electron spin resonance. Biochemistry and Molecular Biology International 47: 979–989.

Krinsky, N. I. 1989. Antioxidant functions of carotenoids. Free Radical Biology and Medicine 7: 617-635.

Kujala, T. S., Loponen, J. M., Klika, K. D. and Pihlaja, K. 2000. Phenolics and betacyanins in red beetroot (*Beta vulgaris*) root: distribution and effect of cold storage on the content of total phenolics and three individual compounds. Journal of Agricultural and Food Chemistry 48: 5338-5342.

Lin, J.-Y. and Tang, C.-Y. 2007. Determination of total phenolic and flavonoid contents in selected fruits and vegetables, as well as their stimulatory effects in mouse splenocyte proliferation. Food Chemistry 101: 140-147.

McDermott, J.H. 2000. Antioxidant nutrients: current dietary recommendations and research update. Journal of the American Pharmacists Association 40: 785–799.

Middleton, E. Jr., Kandaswami, C. and Theoharides, T. H. 2000. The effects of plant flavonoids on mammalian cells: implications for inflammation, heart disease, and cancer. Pharmacological Reviews 52: 673–751.

Noda, Y., Kneyuki, T., Igarashi, K., Mori, A. and Packer, L. 2000. Antioxidant activity of nasunin, an anthocyanin in eggplant peels. Toxicology 148: 119–123.

Oomah, B. D. and Mazza, G. 1996. Flavonoids and antioxidant activities in buckwheat. Journal of Agricultural and Food Chemistry 44: 1746-1750.

Prior, R. L. 2003. Fruits and vegetables in the prevention

of cellular oxidative damage. American Journal of Clinical Nutrition 78: 570S–578S.

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.

Robards, K. and Antolovich, M. 1997. Analytical chemistry of fruit bioflavonoids – A review. Analyst 122:11R-34R.

Roldan-Gutierrez, J. M. and Luque de Castro, M. D. 2007. Lycopene: the need for better methods for characterization and determination. Trends in Analytical Chemistry 26: 163-170.

Saleem, A., Husheem, M., Harkonen, P. and Pihlaja, K. 2002. Inhibition of cancer cell growth by crude extract and the phenolics of *Terminalia chebula* retz. fruit. Journal of Ethno-pharmacology 81: 327–336.

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.

Stafford, H. A. 1994. Anthocyanins and betalains: evolution of the mutually exclusive pathways. Plant Science 101: 91-98.

Vinson, J. A., Hao, Y., Su, X. and Zubik, L. 1998. Phenol antioxidant quantity and quality in foods: vegetables. Journal of Agricultural and Food Chemistry 46: 3630-3634.

Wende, G., Waldron, K. W., Smith, A. C., and Brett, C. T. 1999. Developmental changes in cell-wall ferulate and dehydrodiferulates in sugar beet. Phytochemistry 52: 819-827.

Zhang, Y., Vareed, S. K. and Nair, M. G. 2005. Human tumor cell growth inhibition by nontoxic anthocyanidins, the pigments in fruits and vegetables. Life Sciences 76: 1465–1472.