

Evaluation of the antioxidant activity of asparagus, broccoli and their juices

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Abstract

Antioxidant activity of asparagus, broccoli and their juices was evaluated using 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) and β -carotene bleaching assays. Asparagus showed greater antioxidant activity than broccoli. Asparagus juice also had greater antioxidant activity than broccoli juice. Methanol and acetone extracts of asparagus and broccoli had significantly greater antioxidant activity than their water extracts. Asparagus and broccoli extracts, as well as their juices, showed no significant difference in total phenolics content. However, asparagus contained more flavonoids than broccoli. The antioxidant activity of asparagus and broccoli extracts demonstrated a linear relationship with their flavonoid content.

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1. Introduction

Asparagus and broccoli are vegetables with high antioxidant activity. Antioxidants can scavenge free radicals and protect the human body from oxidative stress, which is the main cause of some cancers and heart diseases (Verlan-gieri, Kapeghian, el-Dean, & Bush, 1985). Asparagus showed one of the greatest antioxidant activities in 43 vegetables (Tsushida, Suzuki, & Kurogi, 1994). The antioxidant activity of broccoli was ranked as the second in 10 common vegetables (Chu, Sun, Wu, & Liu, 2002) and the sixth in 22 vegetables (Cao, Sofic, & Prior, 1996). The per capita consumption of broccoli and asparagus in the USA was ranked as the 12th and 19th, respectively, among the 23 vegetables investigated (Vinson, Hao, Su, & Zubik, 1998). Asparagus and broccoli are mainly consumed fresh;

however, asparagus deteriorates quickly after harvest and is hard to store, thus asparagus juice can increase the total utilization value of asparagus. Asparagus juice and broccoli juice are possible products of asparagus and broccoli, but little research has been reported on these two juices. Several studies have been reported separately either on the antioxidant activity of asparagus (Rodriguez et al., 2005; Tsushida et al., 1994) or broccoli (Cao et al., 1996; Chu et al., 2002; Eberhardt, Kobria, Keck, Juvik, & Jafferh, 2005; Kaur & Kapoor, 2002; Ou, Huang, Hampsch-Woodill, Flanagan, & Deemer, 2002; Plumb, Price, Rhodes, & Williamson, 1997; Proteggente et al., 2002), but only a few reports has been found to compare the antioxidant activity of these two vegetables (Vinson et al., 1998; Wu et al., 2004). The objectives of our research were: (1) to compare the antioxidant activity of asparagus and broccoli, as well as their juices, using DPPH, ABTS and β -carotene bleaching assays; (2) to find out the proper solvent to extract antioxidants of asparagus and broccoli since antioxidant activity could be affected by the extracting solvents (Moure et al., 2000; Sun & Ho, 2005).

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2. Materials and methods

2.1. Materials

Fresh asparagus (*Asparagus officinalis*) and broccoli (*Brassica oleracea*) were purchased from a local market. 2,2'-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS), 6-hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox), Folin–Ciocalteu reagent, 2-aminoethyl diphenylborinate, β -carotene and linoleic acid were purchased from Sigma–Aldrich Chemical Company (St. Louis, MO, USA). Other reagents were obtained from Fisher Scientific (Springfield, NJ, USA).

2.2. Preparation of samples

Asparagus and broccoli were chopped to small pieces and macerated in a food processor (Hamilton Beach/Proctor-Silex Inc., Southern Pines, NC, USA). The macerate (10 g) was homogenized with 10 ml methanol, acetone or water for 1 min and centrifuged at 26,712g for 15 min at 4 °C in a Beckman J2-HS centrifuge (Beckman, Palo Alto, CA, USA). The clear supernatant was transferred to a plastic bottle and stored at –70 °C until analysis. Asparagus or broccoli macerate was wrapped in three-layers of cheese cloth and hand-pressed to prepare the juice. The crude juice was centrifuged at 26,712g for 15 min and the clear supernatant was collected for analysis.

2.3. Analysis of total phenolic content

Total phenolic content of the samples was measured using Folin–Ciocalteu reagent (Velioglu, Mazza, Gao, & Oomah, 1998). Folin–Ciocalteu reagent was diluted by 10 times using deionized water. The diluted reagent (0.75 ml) was mixed with 0.1 ml sample and held at room temperature for 5 min. Then 0.75 ml of 2% sodium carbonate solution was added. After 15 min of incubation at room temperature, the absorbance of the solution was determined at 750 nm by an Ultrospec 4000 UV/vis spectrophotometer (Pharmacia Biotech, Cambridge, England). Catechin was used as a standard.

2.4. Analysis of total flavonoid content

Asparagus and broccoli sample (0.1 ml) was mixed with 0.9 ml water and 0.1 ml of 1% 2-aminoethyl diphenylborinate (Oomah & Mazza, 1996). The absorbance of the solution was determined at 404 nm using a spectrophotometer. Rutin was used as a standard.

2.5. Measurement of antioxidant activity by DPPH and ABTS methods

Various amount of sample were added to 1 ml DPPH solution and the absorbance of DPPH reagent was deter-

mined at 515 nm after 30 min of incubation (Brand-Williams, Cuvelier, & Berset, 1995). The inhibition percentage of the absorbance was calculated as follows:

$$\text{Inhibition \%} = (\text{Abs}_{t=0} - \text{Abs}_{t=30 \text{ min}}) / \text{Abs}_{t=0} \times 100$$

$\text{Abs}_{t=0 \text{ min}}$ was the absorbance of DPPH at time 0. $\text{Abs}_{t=30 \text{ min}}$ was the absorbance of DPPH after 30 min of incubation.

The antioxidant activity of the sample was expressed as Trolox equivalent, which was the ratio between the slope of the sample's regression line (the inhibition % versus amount of sample) and that of Trolox. For the ABTS assay, various amount of sample were mixed with 1 ml ABTS solution and the absorbance was determined at 734 nm after 10 min of incubation at room temperature (Re et al., 1999). The antioxidant activity was expressed as Trolox equivalent as described for the DPPH method.

2.6. Determination of antioxidant activity by β -carotene bleaching method

Twenty milligrams of linoleic acid and 200 mg of Tween 40 were mixed with β -carotene solution. Sample (0.2 ml) was added to 5 ml β -carotene/linoleic acid emulsion and incubated at 50 °C. The absorbance of the emulsion was determined at 470 nm every 15 min until 120 min. Control contained 0.2 ml water and 5 ml β -carotene/linoleic acid emulsion. Antioxidant activity coefficient (AAC) was calculated as follows (Moure et al., 2000):

$$\text{AAC} = (A_{A(120)} - A_{C(120)}) / (A_{C(0)} - A_{C(120)}) \times 1000$$

where $A_{A(120)}$ is the absorbance of the sample at $t = 120$ min, $A_{C(120)}$ is the absorbance of the control at $t = 120$ min, and $A_{C(0)}$ is the absorbance of the control at $t = 0$ min.

Methods for determining antioxidant activity can be divided into three types based on their chemical mechanism: (1) hydrogen atom transfer (HAT) based assay, (2) electron transfer (ET) based assay, (3) other assays (Huang, Ou, & Prior, 2005; Prior, Wu, & Schaich, 2005). DPPH and ABTS method are both HAT and ET based methods and showed consistent results to measure the antioxidant activity of asparagus (Sun, Tang, & Powers, 2005). β -Carotene bleaching assay is a HAT based method. These three methods were thus chosen in the present study.

2.7. Statistical analysis

Each experiment was performed in triplicate. Student's t -test was used to compare the significant difference for total phenolics content or total flavonoid content of asparagus juice and broccoli juice. Analysis of variance (two-factor and three-factor) and multiple comparisons (Fisher's least-significant-difference test) were used to compare the antioxidant activity of asparagus and broccoli and the critical value was set at $\alpha = 0.05$ (Zar, 1996). Two-factor analysis of variance (ANOVA) was used to compare the total

phenolics content or total flavonoid content of asparagus and broccoli extracts, and the same analysis was used to compare the antioxidant activity of asparagus and broccoli extracts determined by each method.

To facilitate the comparison of antioxidant activity, the data from each method were transformed to a relative antioxidant activity index (RAAI: %) by assuming the maximum value in each method as 100% and the others expressed as percentage of the maximum value (see results). To get a trend of the data combining all the three methods, a three-factor ANOVA (vegetable, solvent and antioxidant activity analysis method) was used to compare the antioxidant activity of different solvent extracts and the antioxidant activity of the asparagus and broccoli determined by three methods. A two-factor ANOVA was conducted to compare the antioxidant activity of the two vegetable juices evaluated by the three methods. Regression was conducted to investigate if there was a relationship between antioxidant activity and the content of phenolics/flavonoid. All the statistics were performed with SPSS.

3. Results

3.1. Total phenolics and total flavonoid content

The phenolic contents and flavonoid contents of asparagus and broccoli are shown in Table 1. For total phenolic content, there was no significant difference between asparagus and broccoli or extracts using different extracting solvents (two-factor ANOVA, $P > 0.05$). Flavonoid content of asparagus was higher than broccoli, and methanol and acetone extracted significantly more flavonoids than water (two-factor ANOVA, $P < 0.05$).

3.2. Antioxidant activity

Measured by DPPH method (Table 2), the antioxidant activity of asparagus was significantly higher than broccoli, and the antioxidant activity of methanol extracts were higher than acetone and water extracts (two-factor ANOVA, $P < 0.05$). As measured by the ABTS method, antioxidant activity of asparagus and broccoli did not show significant differences (Table 3), and antioxidant activity of extracts using the three solvents did not show significant difference either (two-factor ANOVA,

Table 1
Total phenolics and total flavonoid content of asparagus and broccoli extracts

	Total phenolics (mg catechin equivalent/g dry weight)		Total flavonoid (mg rutin equivalent/g dry weight)	
	Asparagus	Broccoli	Asparagus	Broccoli
Acetone extract	5.0 ± 0.5	5.2 ± 0.7	4.7 ± 0.5	1.6 ± 0.6
Methanol extract	4.9 ± 0.7	4.9 ± 0.7	4.2 ± 0.3	0.9 ± 0.4
Water extract	4.9 ± 0.9	4.5 ± 1.2	1.5 ± 0.3	0.4 ± 0.1

Table 2
Antioxidant activity (mmol Trolox equivalent/kg dry weight) of asparagus and broccoli extracts determined by DPPH method

	Asparagus	Broccoli
Acetone extract	15.2 ± 3.0	6.0 ± 2.6
Methanol extract	17.4 ± 4.1	11.5 ± 2.8
Water extract	10.9 ± 2.3	4.8 ± 0.4

Table 3
Antioxidant activity (mmol Trolox equivalent/kg dry weight) of asparagus and broccoli extracts analyzed by ABTS method

	Asparagus	Broccoli
Acetone extract	27.1 ± 9.3	25.9 ± 7.9
Methanol extract	26.4 ± 8.2	26.7 ± 7.7
Water extract	26.2 ± 5.8	25.1 ± 5.1

Table 4
Antioxidant activity coefficient (AAC) for asparagus and broccoli extracts analyzed by β -carotene bleaching method

	Asparagus	Broccoli
Acetone extract	357 ± 50	372 ± 65
Methanol extract	336 ± 83	247 ± 51
Water extract	206 ± 37	331 ± 40

$P > 0.05$). According to the β -carotene bleaching method (Table 4), there was no significant difference in the antioxidant activity of the two vegetables (two-factor ANOVA, $P > 0.05$), but acetone extracts had higher antioxidant activity than those of methanol and water extracts.

Three-factor ANOVA showed that asparagus had significantly higher antioxidant activity than broccoli ($P < 0.05$) and water extracts of asparagus had significantly lower antioxidant activity than acetone and methanol extracts ($P < 0.05$) (Fig. 1), but there was no significant difference between acetone and methanol extracts ($P > 0.05$). No relationship was found between antioxidant activity and total phenolics content of asparagus and broccoli, but there was a strong correlation between antioxidant activity and flavonoid contents of asparagus and broccoli (Fig. 2). Our result was in agreement with two previous reports that the antioxidant activity of asparagus is higher than broccoli measured by the inhibition of lower density lipoprotein oxidation (Vinson et al., 1998) or by the oxygen radical absorbance capacity (Wu et al., 2004).

3.3. Asparagus and broccoli juice

Asparagus juice and broccoli juice showed no significant difference in total phenolics content, but asparagus juice contained more than 3 times flavonoid than broccoli juice (Table 5). Two-factor ANOVA demonstrated that the antioxidant activity (Table 5) of asparagus juice (RAAI: 69.01%) was greater than broccoli juice (RAAI: 55.32%) ($P < 0.05$).

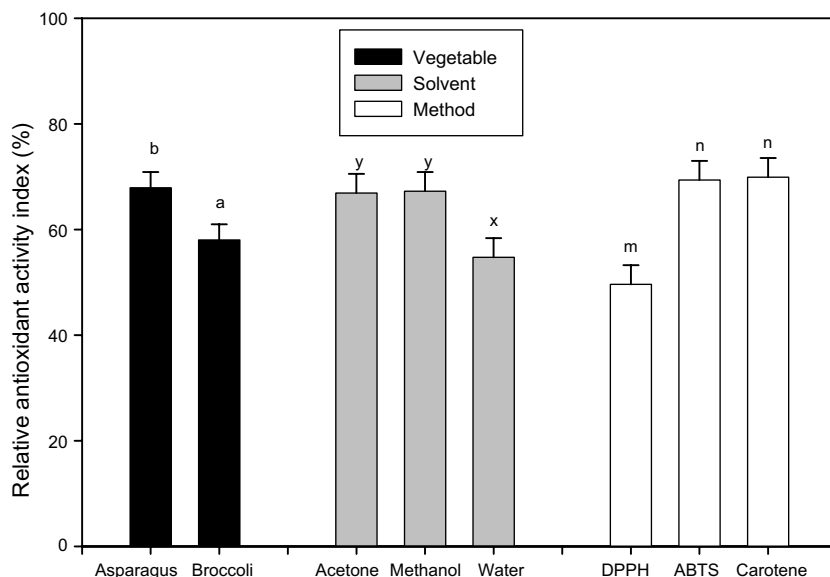


Fig. 1. Relative antioxidant activity index for vegetables, different solvents, and different antioxidant activity assays (mean \pm SE) (data sharing the same letter are not significantly different for the same treatment, $P > 0.05$).

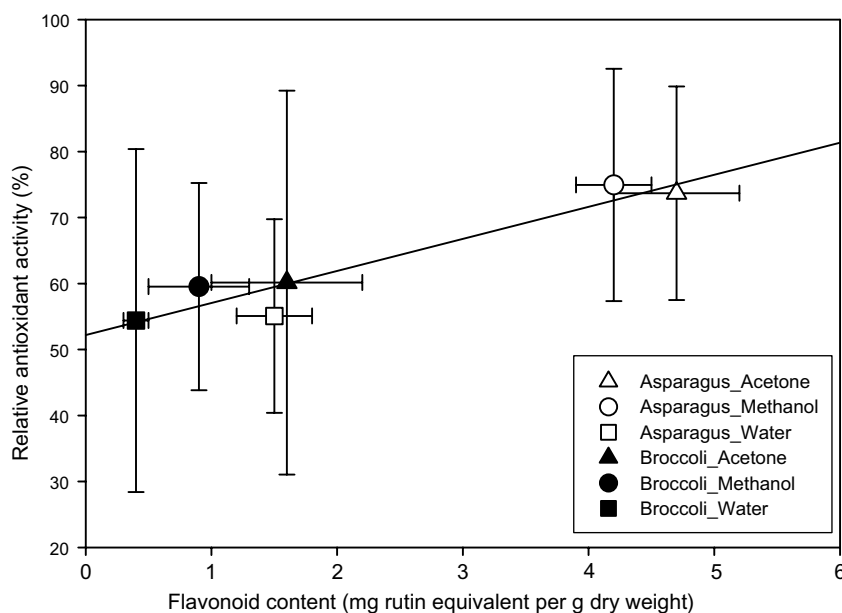


Fig. 2. Relationship between antioxidant activity and flavonoid content in different solvent extracts of asparagus and broccoli ($Y = 4.86X + 52.19$, $R = 0.96$, $P < 0.05$).

Table 5
Phenolics content, flavonoid content, and antioxidant activity of asparagus and broccoli juice determined by three methods

	Asparagus juice	Broccoli juice
Phenolics (mg catechin equivalent/ml)	0.5 ± 0.2^x	0.6 ± 0.1^x
Flavonoid (mg rutin equivalent/ml)	0.3 ± 0.01^y	0.1 ± 0.04^x
DPPH method (mmol Trolox/l juice)	1.2 ± 0.1	0.4 ± 0.2
ABTS method (mmol Trolox/l juice)	3.3 ± 1.7	2.2 ± 0.4
β -Carotene bleaching method (AAC value)	311 ± 190	328 ± 55

Data sharing the same superscript in each row are not significantly different ($P > 0.05$).

4. Discussion

Our results showed that total flavonoid content and the antioxidant activity of asparagus and broccoli had significant correlation (Fig. 2). Therefore, flavonoids are suggested to be a group of key antioxidants in asparagus and broccoli. The major flavonoid antioxidant in asparagus has been reported to be rutin (Tsushida et al., 1994), with the content of 286.5 ± 6.0 mg/kg fresh weight (Makris & Rossiter, 2001). Flavonoid compounds in broccoli include two major compounds (quercetin 3-*O*-sophoroside and kaempferol 3-*O*-sophoroside) and three minor compo-

nents (isoquercitrin, kaempferol 3-*O*-glucoside and a kaempferol diglucoside). The contents of quercetin and kaempferol sophorosides in raw broccoli florets were 65 and 166 mg/kg fresh weight, respectively (Plumb et al., 1997; Price, Casascelli, Colquhoun, & Rhodes, 1998).

From the present experiment, methanol and acetone extracts had higher antioxidant activity than water extracts (Fig. 1), possibly because flavonoids are major antioxidants in both vegetables and are more soluble in methanol and acetone than water. Thus water alone is not a good solvent to extract the antioxidants of asparagus or broccoli. Among the five solvents (water, methanol, ethanol, diethyl ether, acetone) used to extract *Gevuina avellana* hulls, ethanol and methanol extracts showed the highest antioxidant activity while the water extract had the lowest antioxidant activity determined by DPPH method (Moure et al., 2000). Buckwheat was extracted using methanol, acetone, butanol, ethanol, and ethyl acetate (Sun & Ho, 2005). The methanol extract showed the highest antioxidant activity tested by the β -carotene bleaching method and the acetone extract showed the highest antioxidant activity determined by the DPPH method. Thus, the properties of the extracting solvents significantly affected the antioxidant activity of extracts.

The ranking of the antioxidant activity of the samples may vary with the analysis methods. For example, antioxidant activity of tomatoes of several varieties ranked differently when analyzed by DPPH and ABTS methods (Martinez-Valverde, Periago, Provan, & Chesson, 2002). It is common to evaluate the antioxidant activity of plants using several methods to measure various oxidation products. Six methods, which include DPPH, ABTS, total radical-trapping antioxidant parameter assay, *N,N*-dimethyl-*p*-phenyldiamine assay, photochemiluminescence assay and ferric reducing ability of plasma assay, were used to evaluate the antioxidant activity of tea and juices (Schlesier, Harwat, Bohm, & Bitsch, 2002), and blackcurrant juice showed the greatest antioxidant activity among the samples analyzed by all the methods, but the results of the other three samples (tea, apple juice and tomato juice) were different among methods. The authors therefore strongly suggested that, when analyzing the antioxidant activity of antioxidants, it is better to use at least two methods due to the differences between the test systems (Schlesier et al., 2002). Recently, it has been appreciated that there is no simple universal method by which antioxidant activity can be measured accurately and quantitatively (Prior et al., 2005). In our experiment, we used three common methods (DPPH, ABTS, and β -carotene bleaching) to analyze the antioxidant activity of asparagus, broccoli extracts and their juices. The mechanism for each analysis is different. Therefore, the ranking of the antioxidant activity of the two vegetables or different solvent extracts were not the same for these three assays. DPPH and ABTS method measure the ability of antioxidants to scavenge the free radicals, but the free radicals are produced in different way. DPPH \cdot is a free radical reagent relatively stable and ready for use; ABTS \cdot was pro-

duced by the reaction among ABTS, potassium persulfate and H₂O₂ (Re et al., 1999). In the β -carotene bleaching assay, β -carotene was oxidized by the free radicals produced from oxidized linoleic acid, which more closely simulates a real food system compared to DPPH and ABTS methods. Nevertheless, the relative antioxidant activity index (RAAI: %) developed to compare the antioxidant activity of samples using data from all the three methods clearly showed that asparagus and asparagus juice had relatively higher antioxidant activity than broccoli and broccoli juice, and methanol and acetone extracts were greater than water extracts in antioxidant activity (Fig. 1). Despite various mechanisms of the methods, combined results of these in vitro assays have given us an idea of the relative antioxidant activity of different vegetable products and solvent extracts.

In summary, asparagus had greater antioxidant activity than broccoli, and methanol and acetone extracts showed greater antioxidant activity than water extract. There was no significant difference in total phenolics content between asparagus and broccoli, but asparagus had higher contents of flavonoid than broccoli. The antioxidant activity demonstrated significant relationship with flavonoid content in asparagus and broccoli. It is recommended to use more than one method to evaluate the antioxidant activity of asparagus and broccoli as each assay has its own mechanism.

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References

- Brand-Williams, W., Cuvelier, M. E., & Berset, C. (1995). Use of a free-radical method to evaluate antioxidant activity. *Food Science and Technology—Lebensmittel-Wissenschaft und Technologie*, 28(1), 25–30.
- Cao, G. H., Sofic, E., & Prior, R. L. (1996). Antioxidant capacity of tea and common vegetables. *Journal of Agricultural and Food Chemistry*, 44(11), 3426–3431.
- Chu, Y., Sun, J., Wu, X., & Liu, R. (2002). Antioxidant and antiproliferative activities of common vegetables. *Journal of Agricultural and Food Chemistry*, 50(23), 6910–9616.
- Eberhardt, M. V., Kobria, K., Keck, A.-S., Juvik, J. A., & Jefferth, E. H. (2005). Correlation analyses of phytochemical composition, chemical, and cellular measures of antioxidant activity of broccoli (*Brassica oleracea* L. Var. italica). *Journal of Agricultural and Food Chemistry*, 53(19), 7421–7431.
- Huang, D. J., Ou, B. X., & Prior, R. L. (2005). The chemistry behind antioxidant capacity assays. *Journal of Agricultural and Food Chemistry*, 53(6), 1841–1856.
- Kaur, C., & Kapoor, H. C. (2002). Anti-oxidant activity and total phenolic content of some Asian vegetables. *International Journal of Food Science and Technology*, 37(2), 153–161.
- Makris, D. P., & Rossiter, J. T. (2001). Domestic processing of onion bulbs (*Allium cepa*) and asparagus spears (*Asparagus officinalis*): effect on flavonol content and antioxidant status. *Journal of Agricultural and Food Chemistry*, 49(7), 3216–3222.
- Martinez-Valverde, I., Periago, M. J., Provan, G., & Chesson, A. (2002). Phenolic compounds, lycopene and antioxidant activity in commercial

- varieties of tomato (*Lycopersicon esculentum*). *Journal of the Science of Food and Agriculture*, 82(3), 323–330.
- Moure, A., Franco, D., Sineiro, J., Dominguez, H., Nunez, M. J., & Lema, J. M. (2000). Evaluation of extracts from *Gevuina avellana* hulls as antioxidants. *Journal of Agricultural and Food Chemistry*, 48(9), 3890–3897.
- Oomah, B. D., & Mazza, G. (1996). Flavonoids and antioxidative activities in buckwheat. *Journal of Agricultural and Food Chemistry*, 44(7), 1746–1750.
- Ou, B. X., Huang, D. J., Hampsch-Woodill, M., Flanagan, J. A., & Deemer, E. K. (2002). Analysis of antioxidant activities of common vegetables employing oxygen radical absorbance capacity (ORAC) and ferric reducing antioxidant power (FRAP) assays: a comparative study. *Journal of Agricultural and Food Chemistry*, 50(11), 3122–3128.
- Plumb, G. W., Price, K. R., Rhodes, M. J. C., & Williamson, G. (1997). Antioxidant properties of the major polyphenolic compounds in broccoli. *Free Radical Research*, 27(4), 429–435.
- Price, K. R., Casuscelli, F., Colquhoun, I. J., & Rhodes, M. J. C. (1998). Composition and content of flavonol glycosides in broccoli florets (*Brassica oleracea*) and their fate during cooking. *Journal of the Science of Food and Agriculture*, 77(4), 468–472.
- Prior, R. L., Wu, X. L., & Schaich, K. (2005). Standardized methods for the determination of antioxidant capacity and phenolics in foods and dietary supplements. *Journal of Agricultural and Food Chemistry*, 53(10), 4290–4302.
- Proteggente, A. R., Pannala, A. S., Paganga, G., Van Buren, L., Wagner, E., Wiseman, S., et al. (2002). The antioxidant activity of regularly consumed fruit and vegetables reflects their phenolic and vitamin C composition. *Free Radical Research*, 36(2), 217–233.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., & Rice-Evans, C. (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radical Biology and Medicine*, 26(9–10), 1231–1237.
- Rodriguez, R., Jaramillo, S., Rodriguez, G., Espejo, J. A., Guillen, R., Fernandez-Bolanos, J., et al. (2005). Antioxidant activity of ethanolic extracts from several asparagus cultivars. *Journal of Agricultural and Food Chemistry*, 53(13), 5212–5217.
- Schlesier, K., Harwat, M., Bohm, V., & Bitsch, R. (2002). Assessment of antioxidant activity by using different in vitro methods. *Free Radical Research*, 36(2), 177–187.
- Sun, T., & Ho, C. T. (2005). Antioxidant activities of buckwheat extracts. *Food Chemistry*, 90(4), 743–749.
- Sun, T., Tang, J., & Powers, J. R. (2005). Effect of pectolytic enzyme preparations on the phenolic composition and antioxidant activity of asparagus juice. *Journal of Agricultural and Food Chemistry*, 53(1), 42–48.
- Tsushida, T., Suzuki, M., & Kurogi, M. (1994). Evaluation of antioxidant activity of vegetable extracts and determination of some active compounds. *Journal of the Japanese Society for Food Science and Technology—Nippon Shokuhin Kagaku Kogaku Kaishi*, 41(9), 611–618.
- 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(10), 4113–4117.
- Verlangieri, A. J., Kapeghian, J. C., el-Dean, S., & Bush, M. (1985). Fruit and vegetable consumption and cardiovascular mortality. *Medical Hypotheses*, 16(1), 7–15.
- Vinson, J. A., Hao, Y., Su, X. H., & Zubik, L. (1998). Phenol antioxidant quantity and quality in foods: vegetables. *Journal of Agricultural and Food Chemistry*, 46(9), 3630–3634.
- Wu, X. L., Beecher, G. R., Holden, J. M., Haytowitz, D. B., Gebhardt, S. E., & Prior, R. L. (2004). Lipophilic and hydrophilic antioxidant capacities of common foods in the United States. *Journal of Agricultural and Food Chemistry*, 52(12), 4026–4037.
- Zar, J. H. (1996). *Biostatistical analysis* (3 ed.). Upper Saddle River, NJ: Prentice Hall Inc.